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L'ANTAGONISME DE CERTAINS MICRO-ORGANISMES ENVERS *CORYNEBACTERIUM SEPEDONICUM*[1]

PAR CHAMPLAIN PERRAULT[2]

Sommaire

La croissance de *Corynebacterium sepedonicum* (Spieck. & Kott.) Skaptason et Burkholder sur milieu gélosé est entravée par un bon nombre de micro-organismes isolés de tubercules de pommes de terre en putréfaction. Parmi ceux-ci on a observé l'influence antibiotique de dix cultures bactériennes différentes, quatre *Actinomyces* sp., quatre *Penicillium* sp. et un *Chaetomium* sp. Une culture bactérienne et un *Actinomyces* sp. effectuèrent une lyse de *C. sepedonicum*, tandis que trois autres micro-organismes favorisèrent sa croissance.

Introduction

Au cours des études que l'on a poursuivies aux fins de déterminer l'importance des nombreux organismes associés à *Corynebacterium sepedonicum* dans les tubercules de pommes de terre atteints de pourriture, un certain nombre d'entre eux semblaient exercer un antagonisme envers l'agent pathogène de la pourriture du cerne, communément désigné sous le nom de flétrissure bactérienne.

Burkholder (2), en 1938, a rapporté que les colonies de *C. sepedonicum* sur gélose se développent plus rapidement autour de certains agents contaminants. Snieszko et Bonde (3), en 1943, ont attribué une certaine influence toxique aux organismes secondaires observés en culture parmi les colonies de *C. sepedonicum*. Brown et Boyle (1), en 1944, ont obtenu des indications à l'effet que cet agent pathogène est sensible à l'action de la pénicilline.

Parmi les antagonistes que l'on a isolés, les bactéries prédominent. Il y a aussi quelques *Actinomyces*, des *Penicillium* et autres champignons que l'on désigne temporairement par leurs numéros de culture en attendant de les identifier et de déterminer leur importance dans leur association avec *C. sepedonicum*.

Partie Expérimentale

Technique des Essais

Les cultures, à l'exception de *C. sepedonicum*, sont maintenues sur milieu gélosé ordinaire à base de pommes de terre additionné de 15 g. de dextrose,

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[2] *Chef, Laboratoire Fédéral de Phytopathologie, Ste-Anne-de-la-Pocatière (Qué).*

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tandis que *C. sepedonicum* est cultivé sur le même milieu auquel on a ajouté 1 g. d'extrait de levure Bacto et 1 g. de phosphate dibasique de sodium.

Des tubes contenant 15 ml. de ce milieu à l'état liquide ont été ensemencés avec *C. sepedonicum* en suspension dans de l'eau stérilisée. Les tubes ainsi ensemencés ont été versés dans des boîtes de Petri de 90 mm. de diamètre que l'on a conservées à la température du laboratoire. Après une journée d'incubation, les boîtes ont été ensemencées au centre, à l'aide d'une aiguille, avec un de chaque organisme à l'essai. Les cultures qui ont servi d'inoculum étaient âgées de dix jours, tandis que celles de *C. sepedonicum* qui ont servi à la préparation de la suspension utilisée dans l'ensemencement des boîtes de Petri étaient âgées de douze jours.

Résultats

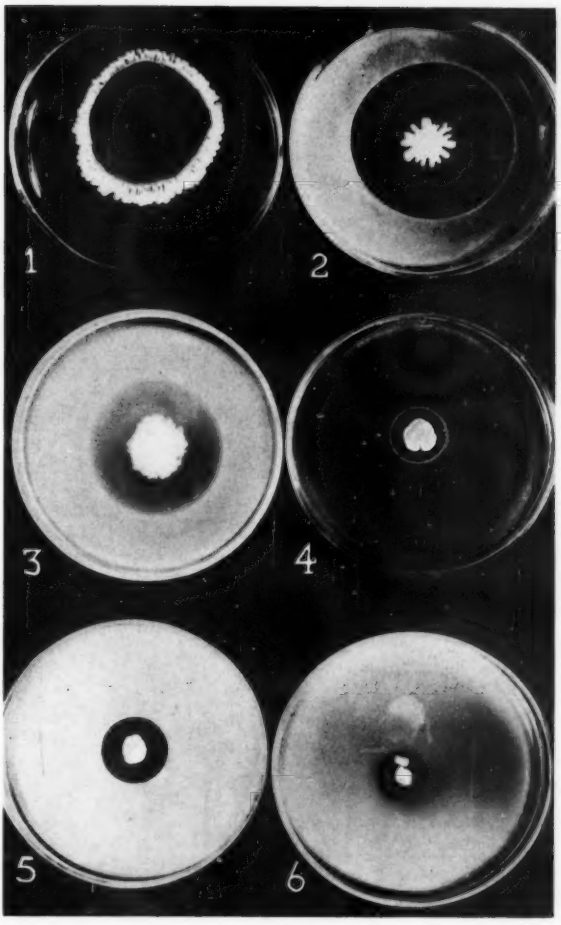
Une des bactéries, le numéro 200, s'est développée rapidement et a couvert la surface de l'agar, empêchant par le fait même toute croissance de *C. sepedonicum*. Neuf autres bactéries se sont montrées antagonistes à *C. sepedonicum*, tandis qu'une autre, numéro 196, a semblé quelque peu favoriser sa croissance.

Lors de son isolation dans la boîte de Petri, où il poussa accidentellement, le numéro 200 a produit une lyse de *C. sepedonicum*. La culture 239, grâce à son expansion et à la diffusion rapide de sa substance antibiotique, a empêché toute croissance de *C. sepedonicum* dans la boîte de Petri (Fig. 1).

La substance produite par la culture 124 s'est diffusée rapidement dans le milieu gélosé et a stimulé quelque peu la croissance de *C. sepedonicum* à la marge extérieure de la zone hyaline (Fig. 2). Ce phénomène est plus accentué avec *Bacillus subtilis* qui a produit aussi une substance antibiotique (Fig. 3). Certaines bactéries, entre autres les numéros 118, 188, 223 et 241, ont également démontré un puissant antagonisme envers *C. sepedonicum*. Quelques-unes, malgré leur expansion excessivement lente et limitée sur le milieu gélosé, ont exercé une influence antibiotique aussi prononcée que d'autres dont la croissance a été plus rapide et abondante.

Au nombre des *Actinomyces* qui ont produit des substances antibiotiques mentionnons *A. scabies* et les espèces désignées par les numéros 189, 235 et 238. *A. scabies* a produit une zone hyaline bien définie autour de laquelle il y a eu, comme pour *Bacillus subtilis* et autres micro-organismes, croissance abondante de *C. sepedonicum*, conséquence d'une stimulation attribuée à une faible concentration de la substance antibiotique. Au delà de cette zone de croissance intensive l'on a observé une autre sphère de cinq à six mm. de largeur, probablement due à l'inégalité du fond de la boîte de Petri (Fig. 4). Les *Actinomyces* sp. 189 et 238 ont exercé une action antibiotique semblable à *A. scabies*. Cependant, la stimulation que l'on observe aux confins de la zone hyaline formée par ce dernier n'est pas aussi évidente avec *Actinomyces* 238 et nulle avec *Actinomyces* 189 (Fig. 5 et 6). *Actinomyces* sp. 235, en plus d'avoir produit la substance antibiotique la plus puissante des quatre organismes du même genre, a exercé une action lytique sur *C. sepedonicum*. On

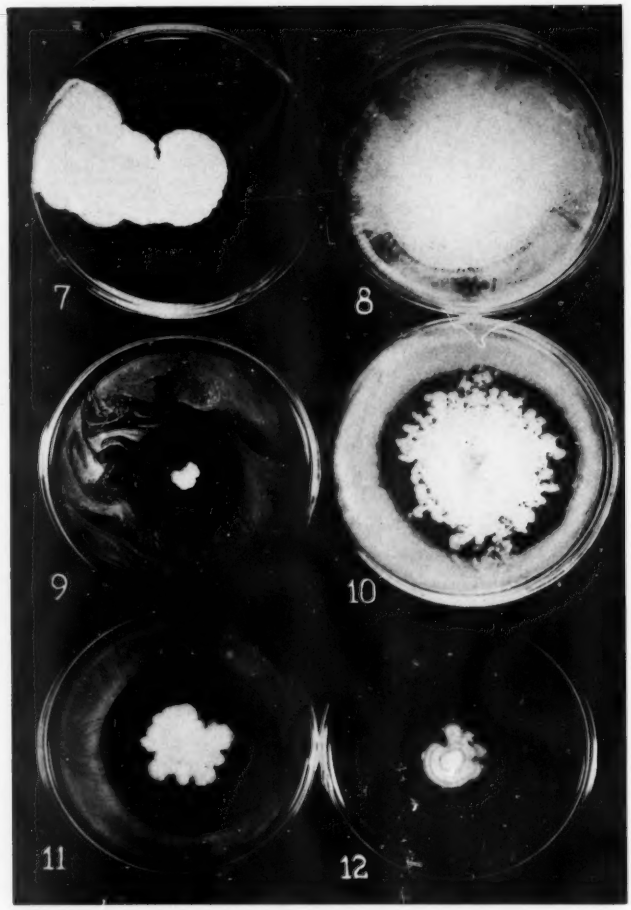
PLANCHE I



Influence antibiotique des micro-organismes sur Corynebacterium sepedonicum.

FIG. 1. *Bactérie 239 n'a permis aucune croissance de l'agent pathogène.* FIG. 2. *Bactérie 124.* FIG. 3. *Bacillus subtilis.* FIG. 4. *Actinomyces scabies.* FIG. 5. *Actinomyces sp. 238.* FIG. 6. *Actinomyces sp. 189. A noter dans les figures 2, 3, 4, la croissance stimulée à la marge extérieure de la zone inhibitrice.*

PLANCHE II



Influence antibiotique des micro-organismes sur Corynebacterium sepedonicum.

FIG. 7. *Penicillium sp. 234. Une faible ligne de croissance est apparente à la partie supérieure de la boîte de Petri.* FIG. 8. *Penicillium notatum.* FIG. 9. *Actinomyces sp. 235 effectue la lyse de C. sepedonicum.* FIG. 10. *Chaetomium sp. 48.* FIG. 11. *Bactérie 236.* FIG. 12. *Bactérie 180.*

peut voir l'effet de cette lyse en observant de près la figure 9. On y distingue une étroite zone intermédiaire où se produit la dissolution de *C. sepedonicum* entre la zone de croissance de cet organisme et la zone hyaline. *Actinomyces* sp. 34 n'a exercé aucun effet antibiotique, mais au contraire a favorisé la croissance de l'agent pathogène.

Quatre différentes cultures de *Penicillium* ont manifesté, à divers degrés, leur antagonisme envers *C. sepedonicum*. Cependant, le *Penicillium* sp. 234 se révéla le plus antagoniste des quatre et la substance antibiotique qu'il secréta se diffusa très rapidement dans le milieu pour enrayer, presque totalement, la croissance de *C. sepedonicum* (Fig. 7). La lignée de *P. notatum* utilisée n'a eu aucun effet sur l'agent pathogène de la pourriture du cerne dont les colonies se sont développées en abondance sous la croissance mycélienne du champignon. Celui-ci a couvert presque toute la surface du milieu gélosé (Fig. 8). Il est vrai que *P. notatum* avait déjà été repiqué plusieurs fois depuis que nous l'avions en laboratoire, mais *Penicillium* sp. 234, ainsi que les autres, l'avaient été aussi souvent depuis leur isolation.

Parmi les autres champignons que l'on a isolés et qui ont montré un certain antagonisme envers *C. sepedonicum* il y a *Chaetomium* sp. 48 dont la substance antibiotique se révéla aussi puissante que celle de certaines bactéries (Fig. 10).

Discussion et Conclusions

Il est évident qu'un bon nombre d'organismes, isolés de tubercules de pommes de terre atteints de pourriture, sont antagonistes à *Corynebacterium sepedonicum* et que la croissance lente de celui-ci sur gélose favorise l'action antibiotique des premiers. On n'a jamais pu isoler *C. sepedonicum* d'un sol non-stérilisé, même quelques jours après l'y avoir enfoui. Il est probable que la présence de ces micro-organismes et d'une multitude d'autres dans le sol entravent la croissance de *C. sepedonicum* ou produisent une lyse de celui-ci. C'est probablement la principale cause des échecs subis lors des essais d'isolation de cet organisme. Il m'est arrivé quelques fois de ne pouvoir isoler *C. sepedonicum* de tubercules de pommes de terre malades, à cause de la présence de certains organismes secondaires qui devaient sans doute en empêcher la croissance dans les boîtes de Petri.

Il serait intéressant de connaître l'action de chacun de ces organismes envers *C. sepedonicum* dans un sol stérilisé, mais il faudrait au préalable, avoir l'assurance que cet agent pathogène puisse s'y développer ou au moins y vivre durant quelques jours. A ce sujet les résultats négatifs obtenus à la suite d'un essai préliminaire ne concordent pas avec ceux de Snieszko et Bonde (3).

Dans les essais rapportés ici-même, l'influence antibiotique des divers organismes a pu être observée dès la cinquième journée après l'ensemencement, alors que les colonies de la plupart des antagonistes étaient encore petites. Cependant, la dimension des zones d'inhibition est restée sensiblement la même, tandis que celle des colonies antagonistes a continué d'accroitre

pour atteindre des proportions que l'on peut observer dans les figures 1 à 12. Les substances antibiotiques produites par les antagonistes n'ont pas empêché la croissance de la plupart de ces micro-organismes.

Summary

The growth of *C. sepedonicum* in agar media was impeded by several micro-organisms isolated from rotted potato tubers affected with ring rot. Some of these organisms produced antibiotic substances that diffused quite readily through the medium and prevented all growth of the pathogen. Among these organisms there were 10 different bacteria, four *Actinomyces* sp., four *Penicillium* sp., and one *Chaetomium* sp. One bacterial culture and one *Actinomyces* sp. produced a lysis of *C. sepedonicum*, while three other organisms seemed to stimulate its growth.

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STUDIES ON STEAM STERILIZATION OF SOILS

I. SOME EFFECTS ON PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES[1]

By S. N. Malowany[2] and J. D. Newton[3]

Abstract

Effects of steam sterilization and recontamination with original soil, on certain physical, chemical, and biological properties of four Alberta soils were investigated. The soils were Edmonton black loam, Vegreville black fine sandy loam, Gros Ventre brown loam, and Fallis gray silt loam. Certain physical properties were affected by steam sterilization as follows: (1) capillary rise of moisture was greatly retarded in all four soils following sterilization; (2) the percentages of the different separates as determined by ordinary mechanical analysis were not changed significantly by sterilization; (3) water-holding capacity and moisture content at the 'sticky point' were slightly reduced, and, in three of the soils, shrinkage was slightly increased by sterilization, but these effects were of uncertain significance; (4) the pH values of these soils were not changed appreciably by sterilization. The following chemical changes were produced by steam sterilization: (1) water-soluble phosphate was generally greatly increased and water soluble sulphate somewhat increased by sterilization. Easily soluble phosphorus (soluble at pH 3) was increased by about one-third in the two black soils rich in organic matter, but not in the other soils; (2) ammonia accumulated rapidly in the sterilized recontaminated black and brown soils for four to six weeks, and then returned to normal in 8 to 12 weeks. Ammonia accumulated less rapidly and to a smaller degree in the gray soil; (3) nitrification was suppressed for about 6 to 10 weeks in the sterilized recontaminated soils, but was later more active in these soils than in the unsterilized (especially in the black soils rich in organic matter).

Introduction

The study of some physical, chemical, and biological changes brought about by steam sterilization of soils, as reported in this paper, is part of a broader study of the inter-relationships of soil micro-organisms and plant diseases, and of soil micro-organisms and soil fertility. The study of these complicated inter-relationships may be somewhat simplified by first studying the relationships of a few known organisms on sterilized soil devoid of its enormous population. However, the study of effects of known micro-organisms in sterilized soil necessitates a study of effects produced by sterilization, because the sterilized soil may be quite different from the original soil, physically and chemically, and the results obtained may be misleading if the changes due to sterilization are not measured.

In practice, mainly in greenhouses, soils are partially sterilized to get rid of weeds, insects, and other undesirable or disease producing organisms. Complete sterilization is neither strived for nor desirable since there are many organisms in the soil that are beneficial to plant growth because they are

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Contribution from the Department of Soils, University of Alberta, Edmonton, Alta., with financial assistance from the National Research Council of Canada.

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[3] *Professor of Soils, University of Alberta.*

responsible for the decomposition of organic matter and the elaboration of plant nutrients.

Literature dealing with the changes effected when soils are completely sterilized by steam is less abundant than that dealing with partial sterilization. Effects of various heat and chemical treatments in partially sterilized soils have been studied by many workers in England and the United States, and their findings would apply, in varying degrees, to the Alberta soils used in these investigations. However, different soils respond differently to such treatments, and, furthermore, the Alberta soils were sterilized completely rather than only partially.

Darbishire and Russell (7) noted that in steam sterilized soils heated to 120° C. the absorption of oxygen was greatly diminished, but that in partially sterilized soils, heated to 95° C., the absorption was considerably increased. They found that the crop could get from heated soils three times as much nitrogen and twice as much phosphorus and potash as from the unheated soils. They believed that the increased availability of the plant nutrients was connected with the modification of microbial flora, although they recognized that when soils were heated or sterilized by steam, a certain amount of decomposition took place.

Russell and Hutchinson (16) found that heating the soil by steam to 125° C. killed all the organisms present, and caused a production of 5 p.p.m. of ammonia, but after that first production no further change took place. Heating by steam to 98° C., or treating with toluene, increased ammonification greatly for a time, and they concluded that these treatments destroy organisms that are inimical to ammonifying bacteria. The number of bacteria increased greatly following partial sterilization, and this they attributed to the absence of protozoa. They found that heating to 98° C. not only destroyed the nitrifiers, but made the soil temporarily unfit for their development.

Lyon and Bizzell (11) observed that steamed soil, on standing up to three months without plants, steadily decreased in water soluble organic matter, ammonia, and other soluble nitrogenous matter. They found that nitrates remained low, up to 14 weeks in steamed and recontaminated soils, but that ammonia increased. Baldwin (1) reports a consistent increase in acid soluble phosphorus after sterilization. Pickering (14) and Newhall (13) found a lower water-holding capacity in steam sterilized soil.

Practical sterilization is discussed by Bewley (2), Senner (18), and Newhall (13). Newhall concludes that steam sterilization leaves no toxic residues or water-logging and leaves the soil in good tilth.

Methods

The investigation of changes effected by steam sterilization of Alberta soils and subsequent microbial activities following recontamination was divided into two parts: first, the physical and second the chemical–biological changes. Four Alberta soils, Edmonton black loam, Vegreville black fine sandy loam,

Gros Ventre brown loam, and Fallis gray silt loam, were studied. The first two are high in organic matter and the last, very low.

In the study of the physical properties of these soils, the non-sterilized were compared with the sterilized with respect to mechanical analysis, water-holding capacity, shrinkage, sticky point, and capillary power. Since soil moisture relations affect greatly the ultimate productive power of the soil, certain differences in sterilized soils may be due to purely physical factors.

The chemical–biological studies included determinations of acidity, ammonia, nitrates, sulphates, easily soluble phosphorus, and ammonia production by pure cultures.

In the study of physical and biochemical properties, the non-sterilized soils were compared with the sterilized, and with the sterilized–recontaminated also, in the case of the biochemical properties.

For the study of physical properties the soils were sterilized in 1-gal. crocks, and for the study of biochemical properties the soils were placed in Erlenmeyer flasks and incubation was carried on in a dark control chamber at room temperature (23° C. to 27° C.).

Two nitrification experiments were carried out, the first for a period of 12 weeks and the second for 39 weeks. Nitrates were determined frequently throughout these periods, and one flask or tumbler was taken for each determination. Similar plans were followed in the case of the ammonification and sulphofication experiments. Easily soluble phosphorus was determined on the same sample as ammonia. The experiment on ammonia production by pure cultures of *Ophiobolus* and two other fungi, one antagonistic and the other not antagonistic to *Ophiobolus*, was carried out in Erlenmeyer flasks of sterilized Edmonton black soil inoculated with these organisms. The results of these experiments with pure cultures will be published in a subsequent paper together with results of other similar experiments.

Representative samples of the surface six inches of the four soils were obtained, air-dried, put through a 2 mm. sieve, and well mixed. The soils were brought to optimum moisture and allowed to stand for two days or less before sterilizing. Table I shows the optimum moisture used for each soil and a few other physical characteristics of the four soils.

Sterilization was done by steaming the soil at 15 to 17½ lb. pressure (approximately 120° C.) for 45 min. and again next day at the same pressure for 30 min.

After sterilization the soils were cooled and half of the sterile flasks were recontaminated for nitrification, ammonification, and sulphofication experiments.

In all cases where the soil was incubated, the flasks were taken out of the chamber every week or 10 days and brought up to optimum moisture with sterile water. By means of a stirring rod that had been placed in each flask through the cotton plug, the soils were cultivated after being watered.

TABLE I

PHYSICAL CHARACTERS OF THE FOUR ALBERTA SOILS

—	Treatment	Hygroscopic moisture, %	Moisture-holding capacity, %	Optimum moisture, %	Real specific gravity	Sticky point moisture, %
Edmonton black park (loam to silt loam)	Non-sterilized		76		2.34	60
	Sterilized	5.96	72	36	2.40	58
Vegreville black park (sandy loam to fine sandy loam)	Non-sterilized		71		2.51	52
	Sterilized	4.52	71	30	2.54	48
Gros Ventre brown prairie (loam)	Non-sterilized		75		2.32	50
	Sterilized	2.13	74	32	2.33	46
Fallis gray wooded (silt loam)	Non-sterilized		59.5		2.54	33
	Sterilized	1.22	58.5	19	2.51	33

For mechanical analysis, Bouyoucos' hydrometer method (3) was adopted. Moist samples were used rather than dry ones because it was thought that following sterilization drying might affect the soil colloidal content and thereby alter changes induced by steam sterilization. The non-sterilized samples had the same amount of moisture added as the sterilized ones and were kept moist for the same length of time prior to mechanical analysis. Since it is more difficult to weigh accurately moist samples, owing to loss of moisture, a greater analytical error had to be contended with. Otherwise, in the main, the procedure recommended by Bouyoucos was followed. The samples were dispersed by adding water, treating with solutions of sodium oxalate and sodium silicate, and mixing in a mechanical stirrer.

Some seven determinations were made also by the pipette method for comparison with the hydrometer method.

The real specific gravity of these soils (specific gravity of soil particles) was calculated from water displaced in a volumetric flask by 10 gm. of ovendry soil.

Water-holding capacity was found by the funnel method, using 50 gm. of soil. Readings were taken when drainage stopped, and in certain determinations readings were made at 15 min., one hour, and two hours.

The 'sticky point' moisture content of the sterilized and non-sterilized soils was determined by a somewhat arbitrary method. Air-dry 50 gm. samples were moistened with a measured amount of water and the moisture was worked into the soil with a spatula on a glass plate until the sticky point was nearly reached. This was judged by the appearance of wetness. Then the wet soil was kneaded by hand and a little more moisture worked into it, until the point of maximum stickiness was attained as judged by the adhesion to the palm of the hand and the force required to tear away the soil cake after it was squeezed in the hands.

Shrinkage was determined by a modification of Haines' mercury displacement method (8). The soils were brought to optimum moisture content, and half of each was sterilized as previously described. Then both the non-sterilized and the sterilized soils were air-dried and passed through a 100 mesh sieve. Into 50 gm. of soil on a glass plate a measured amount of water was incorporated by means of a spatula, until the sticky point was nearly reached. It was kneaded, shaped by hand, and moistened, more especially at the ends, to prevent cracking. The wet cylinder was weighed and its volume determined by placing it in a large glass test tube, which was then filled with mercury, and levelled off with a microscope slide. The buoyant effect was overcome by placing a perforated rubber stopper below the surface of the mercury. Weight and volume of the soil cylinder were measured at intervals of drying, first at room temperature, later in an oven at 100° C., and finally after drying for two days at 110° C. All data were reduced to the basis of 1 cc. ovendry soil. Some difficulty arises in finding the first volume if the soil cylinder is too wet, as the buoyant force of the mercury compresses the soil and distorts the soil cylinder; also, small bubbles often adhere. But with a little drying these difficulties can be overcome and the volume measured accurately.

Capillary rise of water in the sterilized and unsterilized soils was determined by measuring the rise in glass tubes about $1\frac{1}{8}$ in. in diameter. The soils were prepared, sterilized, and air-dried as previously described. The air-dried soil was then put through a 2 mm. sieve, and the tubes, wrapped at the lower end with cheesecloth, were filled uniformly with the soils. The tubes were set vertically in a pan to which water was added and maintained at a constant level. From the time of addition of water, measurements of capillary rise above the water level in the pan were taken at 5 min., 15 min., 1 hr., 2, 3, 4, 12, and 24 hr. and thereafter every day for 42 days, and at longer intervals up to 61 days from the beginning.

The pH values were determined by the quinhydrone electrode method. One day after sterilization, moist samples of sterilized and unsterilized soil equivalent to 5 gm. of water-free soil were diluted with 20 cc. of distilled water and pH values read at 1, 5, 15, and 30 min.

Water soluble phosphorus was determined colorimetrically on 1 : 5 water extract of soil, by the Parker and Fudge method (15). Moist samples equivalent to 50 gm. water-free soil were shaken for one hour and filtered through Berkefeld filters. Then 25 or 50 cc. portions of filtrate were evaporated with a little magnesium nitrate and ignited to remove organic matter before determining phosphorus. However, in a few cases, for purposes of comparison, the dried filtrates were not ignited.

Easily soluble phosphorus (soluble at pH 3) was determined colorimetrically by Truog's (19) method. Moist samples of soil equivalent to 1 gm. water-free soil were extracted by shaking for one hour with 200 cc. buffered pH 3.0 sulphuric acid solution, adjusted when necessary by the addition of one to seven drops of normal sulphuric acid. The pH values were determined with

the potentiometer before and after extraction. A few soil extracts, after being filtered through paper, were highly coloured and such extracts were cleared by means of activated charcoal. Blue colour was developed with ammonium molybdate–sulphuric acid solution and stannous chloride.

Nitrates were determined colorimetrically on 1 : 5 water extracts of soil by the phenol–disulphonic method as modified by Harper (9).

Ammonia was determined by the McLean and Robinson (12) method of leaching 25 gm. of soil with 500 cc. normal sodium chloride solution, distilling the soil extract with magnesium oxide, and collecting the ammonia in standard acid.

Water soluble sulphates were determined gravimetrically. The 1 : 5 extracts were filtered through Berkefeld filters, evaporated with magnesium nitrate, and ignited to destroy organic matter prior to solution in dilute hydrochloric acid and precipitation as barium sulphate.

Results and Discussion

Mechanical Analysis

Mechanical analysis of 25 samples of soil were made and the results of 16 are represented in Fig. 1. Taking the silt particles limits as 0.002 mm. to 0.05 mm. in diameter, the Edmonton black park soil should be classed as loam to silt loam, the Vegreville black park soil as sandy loam (probably fine sandy loam), the Gros Ventre brown prairie soil as loam, and the Fallis gray wooded soil as silt loam. Differences between the sterilized and unsterilized soils were not obtained by the methods of mechanical analysis used in these experiments.

Water-Holding Capacity

The funnel method of determining water-holding capacity was used and the results indicate that the sterilized soils have about 1% lower water-holding capacity than the unsterilized, but the differences were not obtained in every case and cannot be taken as definitely significant. The water-holding capacities are shown in Table I. Pickering (14) and Newhall (13) report that sterilized soils have a lower water-holding capacity than unsterilized, but that the former hold water for a longer time.

Shrinkage

The results of the shrinkage experiments are represented by Figs. 2 and 3. They show that during the early stages of drying there was a decrease in volume proportional to the amount of water lost. This decrease, represented graphically, is shown by the slant of the curve at an angle of approximately 45° to the abscissa.

There was a sudden change in the direction of the curves in Fallis gray wooded soil from about 0.34 cc. moisture per gram water-free soil up to the last stage of drying; the curves become almost parallel to the base (abscissa).

This stage of drying is accompanied by a loss in moisture that is relatively large in comparison with the loss in volume, and is called 'residual' shrinkage.

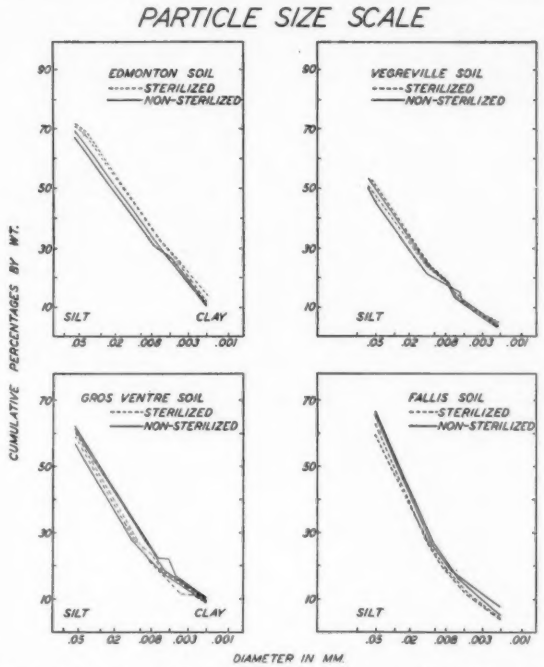


FIG. 1. *Mechanical analysis of non-sterilized and steam sterilized Edmonton and Vegreville black park soils, Gros Ventre brown prairie soil, and Fallis gray wooded soil.*

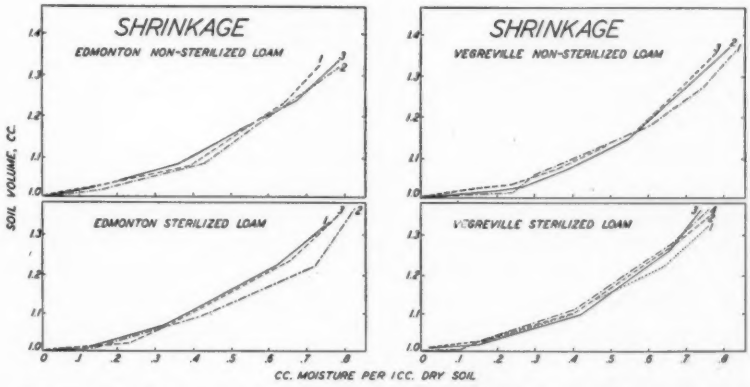


FIG. 2. *Shrinkage of non-sterilized and steam sterilized Edmonton and Vegreville black park soils.*

This residual shrinkage in the unsterilized Fallis soil amounted to approximately 0.02 cc. decrease in volume for 0.35 cc. of moisture lost, and, in the sterilized, 0.025 cc. for 0.33 cc. moisture lost.

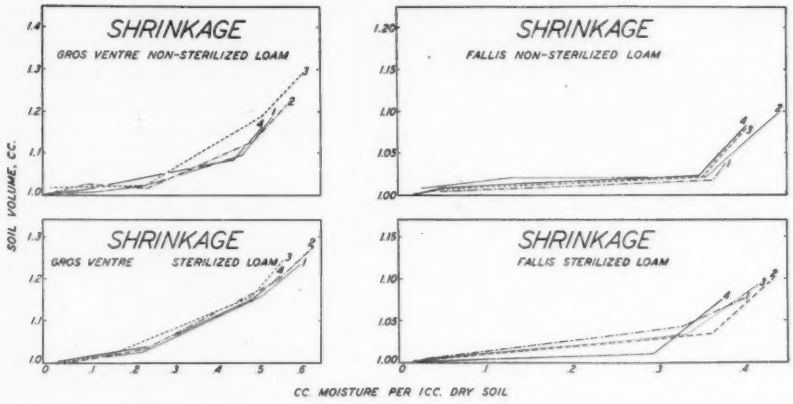


FIG. 3. *Shrinkage of non-sterilized and steam sterilized Gros Ventre brown prairie soil and Fallis gray wooded soil.*

The break between the 'normal shrinkage' and the 'residual' shrinkage is not so sharp in the Gros Ventre brown prairie soil as might have appeared if a determination had been made at about 0.37 cc. moisture content per cc. of dry soil. The volume of soil at 0.24 cc. of moisture, which is well within the region of 'residual' shrinkage, is, in the unsterilized, approximately 1.02 cc. while in the sterilized at the same moisture it is approximately 1.03 to 1.04 cc. Again this is a small difference, but the shrinkage is greater in the sterilized.

The Vegreville soil shows a gradual change instead of a sharp break. This is indicated by the points plotted between 0.1 cc. and 0.65 cc. of moisture. A slightly greater shrinkage in the case of the sterilized as compared with the unsterilized soil, of about the same magnitude as in the former two soils, was obtained below a moisture content of approximately 0.25 cc. The Edmonton soil shows the same trends as the Vegreville soil.

From Figs. 2 and 3 it appears that 'residual' shrinkage is greatest in Edmonton black soil, followed by Vegreville black, and least in Fallis gray wooded. This indicates that soils high in organic matter have greater 'residual' shrinkage than soils low in this respect, and agrees with the findings of Haines (8) who obtained the highest 'residual' shrinkage, 18.2% volume, on drying peat soil below 50.5% moisture, and only 2.2% in Rothamsted loam below 18.7% moisture. The low 'residual' shrinkage of Fallis gray wooded soil indicates a loss of colloidal material from the soil, probably by leaching, since it is a badly leached soil.

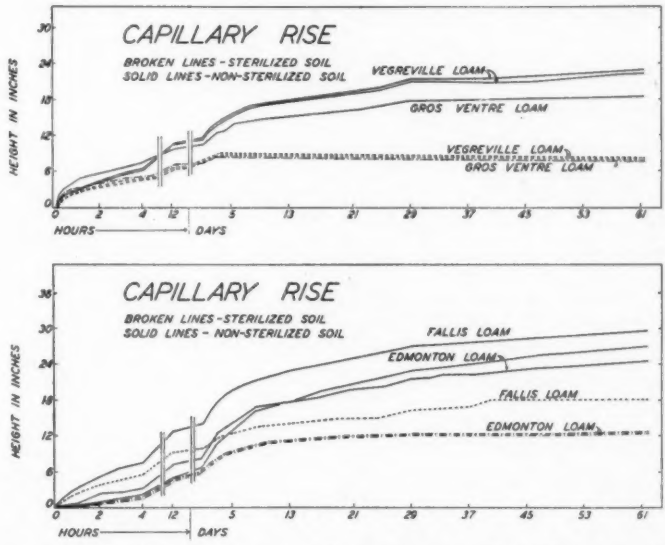
Sticky Point

The 'sticky point' or point of maximum stickiness appears to be reached at a lower moisture content in sterilized soils than in the unsterilized. It was reached when Edmonton unsterilized soil was brought to 60% moisture (air-dry basis) and sterilized soil was brought to 58%. At a given moisture content, approximately 40% moisture, Edmonton sterilized was more sticky than unsterilized soil. Vegreville, Gros Ventre, and Fallis unsterilized attained sticky points at 52, 50, and 33% of moisture.

On cultivating the soils in the flasks incubated for nitrification, it was observed that those that were kept sterile for a long time became quite sticky upon repeated addition of water to maintain weight for optimum moisture, while the unsterilized and the sterilized recontaminated, with the same moisture content, were not inclined to become sticky.

Capillary Rise

Figs. 4 and 5 show the results of the capillary rise experiment. Consistent and definite differences were found. In all cases the capillary rise after the first 12 hr. was greater in the unsterilized soils. Differences became



FIGS. 4 and 5. *Capillary rise in non-sterilized and steam sterilized Edmonton and Vegreville black park soils, Gros Ventre brown prairie soil, and Fallis gray wooded soil.*

apparent even after 15 min. in Fallis and Gros Ventre soils. The experiment was carried on for 58 days and the highest rise in unsterilized soil at the end of the experiment was obtained in Fallis soil, the next highest in Edmonton

soil, and the lowest in Gros Ventre. In the sterilized soils the rise was in the same order, although not as high. After 58 days the sterilization had decreased capillary rise by about 49, 62, 53, and 37%, respectively in the Edmonton, Vegreville, Gros Ventre, and Fallis soils.

The differences in capillary rise of water in the different soils, and between sterilized and unsterilized soils, are probably due mainly to differences in texture, quantity, and character of colloidal material present. The quantity of soluble matter, including soluble organic matter, is definitely increased by steaming. Pickering (14) and Lyon and Bizzell (11) found that water soluble matter was increased by steaming.

pH Values

In this investigation, as shown in Table II, no significant differences were found in pH values, as measured by the quinhydrone electrode, between sterilized and unsterilized soils after the sterilized soils had been autoclaved

TABLE II

HYDROGEN ION CONCENTRATION OF FOUR STERILIZED AND NON-STERILIZED ALBERTA SOILS, EXPRESSED AS pH

(Determined by the quinhydrone electrode)

	Sterilized				Non-sterilized			
	1 min.	5 min.	15 min.	30 min.	1 min.	5 min.	15 min.	30 min.
Edmonton	6.0	6.1	6.1	6.0	6.3	6.1	6.2	6.0
black	6.1	6.1	6.1	6.1	6.3	6.1	6.3	6.0
park	6.0	6.1	6.1	6.1	6.2	6.2	6.1	6.2
Average	6.0	6.1	6.1	6.1	6.3	6.1	6.2	6.1
Vegreville	6.3	6.1	6.1	6.2	6.2	6.2	6.1	6.2
black	6.2	6.1	6.1	6.2	6.2	6.1	6.0	6.2
park	6.2	6.1	6.1	6.2	6.3	6.1	6.2	6.1
Average	6.2	6.1	6.1	6.2	6.2	6.1	6.1	6.2
Gros Ventre	5.8	5.7	5.8	5.8	6.0	5.8	5.9	5.9
brown	5.8	5.8	5.8	5.7	5.9	5.8	5.9	5.9
prairie	5.7	5.7	5.8	5.7	5.8	5.8	5.9	5.9
Average	5.8	5.7	5.8	5.7	5.9	5.8	5.9	5.9
Fallis	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.1
gray	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2
wooded	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2
Average	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2

twice, on successive days, once for three-quarters of an hour and the second time for one-half hour at 120° C. Titratable or total acidity was not determined. Schreiner and Lathrop (17) in 1912 found an increase in titratable acidity when they steam sterilized a soil for three hours at 135° C.

Water Soluble Phosphorus

Water soluble phosphorus was definitely increased in steam sterilized soils, as shown in Table III. In comparison with unsterilized soil, the quantities were about 2.8 times as great in the Edmonton soil, 1.7 times in the Vegreville, 2.1 times in the Gros Ventre, and there was a slight increase in the Fallis soil. Without the ignition of water extracts to destroy organic matter, the quantities of water-soluble phosphorus were found to be approximately half as great in both unsterilized and sterilized soils.

TABLE III

Water soluble phosphorus in non-sterilized and sterilized Alberta soils

(Average p.p.m. phosphorus on water-free basis)

Soil	Non-sterilized			Sterilized		
	No. of determinations	P, p.p.m.	Av. deviation from mean	No. of determinations	P, p.p.m.	Av. deviation from mean
Edmonton black park	12	1.67	.47	18	4.69	.48
Vegreville black park	5	4.17	.22	5	7.01	.20
Gros Ventre brown prairie	4	3.16	.05	4	6.72	.05
Fallis gray wooded	4	1.38	.01	4	1.83	.04
Without ignition						
Vegreville black park	4	2.25	.37	4	3.80	.70
Gros Ventre brown prairie	4	1.00	.05	—	—	—

The increase in water soluble phosphorus content induced by sterilization was large in the soils high in organic matter and relatively small in Fallis soil, which is low in organic matter, suggesting that organic phosphorus compounds are partly broken down or made more soluble and later broken down by ignition.

Schreiner and Lathrop (17) found that steam sterilization increased the solubility of soil constituents, increasing all of the organic constituents that they had isolated from unheated soil, except nucleic acid, which was decomposed.

Easily Soluble Phosphorus (Soluble at pH 3)

The quantities of easily soluble phosphorus in the four Alberta soils (1) unsterilized, (2) sterilized, and (3) sterilized and recontaminated with original soil, throughout an incubation period of 20 weeks following sterilization, are shown in Table IV. In general, greater quantities of easily soluble phosphorus were found in the sterilized and sterilized recontaminated black Edmonton and Vegreville soils than in the unsterilized. In the Gros Ventre

TABLE IV

Easily soluble phosphorus (soluble at pH 3) in non-sterilized, sterilized, and recontaminated sterilized Alberta soils

(Average of duplicates, p.p.m. phosphorus on water-free basis)

Soil	Treatment	Period of incubation after sterilizing soil and setting up experiment									Final av.
		Right after ster.	Days		Weeks						
			3	6	2	4	8	12	16	20	
		Phosphorus, p.p.m., average of duplicates									
Edmonton black park	Non-sterilized	33	—	32	32	37	46	52	49	55	43
	Sterilized and recontaminated	—	—	40	47	57	52	74	75	80	61
	Sterilized	40	—	42	51	56	56	77	70	63	59
Vegreville black park	Non-sterilized	46	—	82	86	69	96	91	86	108	88
	Sterilized and recontaminated	—	—	78	89	93	125	123	103	113	103
	Sterilized	60	—	83	99	83	103	109	104	110	99
Gros Ventre brown prairie	Non-sterilized	56	48	48	46	56	54	98	56	57	58
	Sterilized and recontaminated	—	48	49	43	53	61	107	61	67	61
	Sterilized	62	56	54	48	52	55	82	53	61	59
Fallis gray wooded	Non-sterilized	87	76	83	82	92	100	115	76	100	90
	Sterilized and recontaminated	—	85	86	82	103	102	120	91	105	97
	Sterilized	99	90	91	82	94	106	125	84	101	97

brown soil, which is low in organic matter, the differences were insignificant, and in the Fallis gray soil, which is also low in organic matter, the differences were probably insignificant, because, while close agreement was obtained in reading the duplicates of a single determination, a variation of 10 p.p.m. was often obtained in separate determinations. In the final averages the increases of sterilized over unsterilized were about 37% in the Edmonton soil, 12% in the Vegreville, 8% in the Fallis, and if anything a little more in the sterilized recontaminated.

Increases in soluble phosphorus found right after sterilization must be attributed to changes brought about by steaming. Increases with time of incubation may be attributed to microbiological activity in the unsterilized

and the sterilized recontaminated soils, following remoistening and cultivation, but this is doubtful because the sterilized soils that were not recontaminated also showed an increase in easily soluble phosphorus.

Darbishire and Russell (7) found that plants grown on partially sterilized soil utilized about twice as much phosphorus as plants grown on normal soil, and that the percentage of phosphorus in plants grown on partially sterilized is much higher than on normal soil. Baldwin (1) found more easily soluble phosphorus in sterilized than in unsterilized soil in the greenhouse.

Nitrification

The results of the two nitrification experiments are shown in Figs. 6, 7, 8, and 9. Greatest nitrification occurred in Vegreville and Edmonton soils, less in Gros Ventre, and least of all in Fallis soil; that is to say, the higher the organic matter content of the soil, the greater the nitrification.

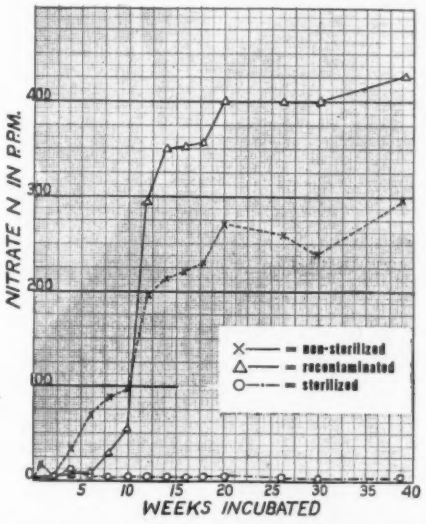


Fig. 6. *Nitrification in non-sterilized, recontaminated sterilized, and sterilized Edmonton black park soil.*

In all cases the nitrate content remained practically constant and generally very low in the sterilized soils throughout the 39 weeks of incubation. Nitrate accumulated in all of the unsterilized soils. Nitrification was slower at first in the sterilized recontaminated soil but after about 10 weeks, nitrate accumulated more rapidly in the sterilized recontaminated than in the unsterilized. The increase in the rate of nitrification in the sterilized recontaminated is evidently related to the ammonia content of the soil, because the ammonia that had accumulated in these soils began to decrease rapidly in most cases at about 10 weeks or a little earlier.

These results indicate that either the nitrifying organisms (*Nitrosomonas* and *Nitrobacter*) take a long time to become active when re-introduced into

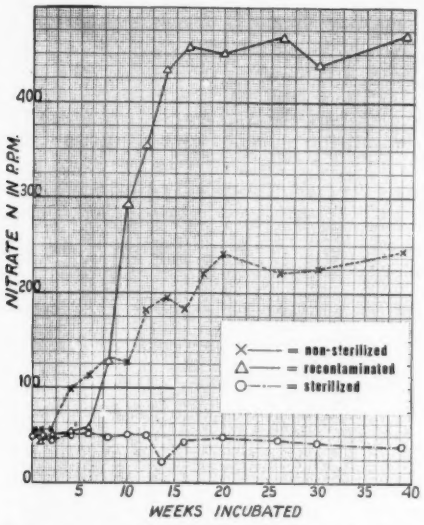


FIG. 7. *Nitrification in non-sterilized, recontaminated sterilized, and sterilized Vegreville black park soil.*

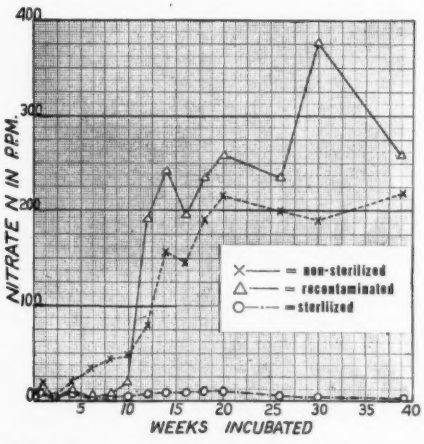


FIG. 8. *Nitrification in non-sterilized, recontaminated sterilized, and sterilized Gros Ventre brown prairie soil.*

steam sterilized soil, or that sterilization in some way alters the chemical composition of the soil, making it unfavorable for their development until other micro-organisms have had time to overcome the inhibitory factors.

Schreiner and Lathrop (17) found an increase in a large number of organic nitrogen compounds following steam sterilization, and this may account, in part at least, for the greater nitrification in the recontaminated soils. Presumably the same micro-organisms as were present in the unsterilized soil are active, though certain groups may develop more rapidly for a time because of the altered chemical and physical conditions in sterilized soils.

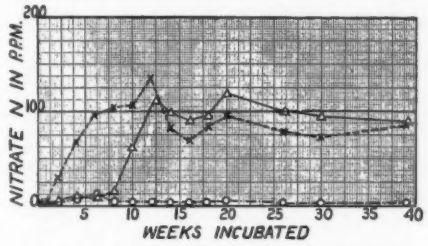


FIG. 9. *Nitrification in non-sterilized, recontaminated sterilized, and sterilized Fallis gray wooded soil; X —— X = non-sterilized; △ —— △ = recontaminated; O —·—·— O = sterilized.*

AMMONIFICATION

The greatest ammonification or accumulation of ammonia occurred in the sterilized soil recontaminated with original soil, as shown in Figs. 10 to 13. In the first four to six weeks of incubation, a steady increase in ammonia took place in three out of four of the recontaminated soils, rising to a high peak,

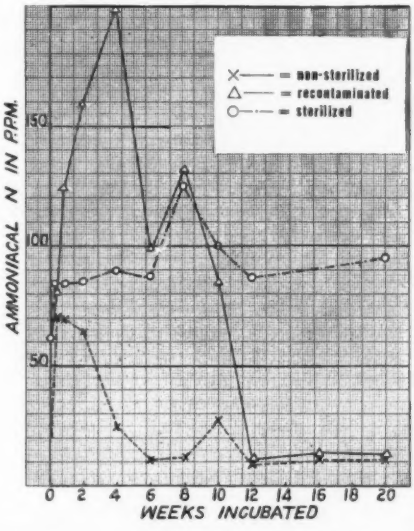


FIG. 10. *Ammonification in non-sterilized, recontaminated sterilized, and sterilized Edmonton black park soils.*

as may be seen on Figs. 10, 11, and 12. This increase was followed by a rapid return to about the same low level as that of the unsterilized, at about 12

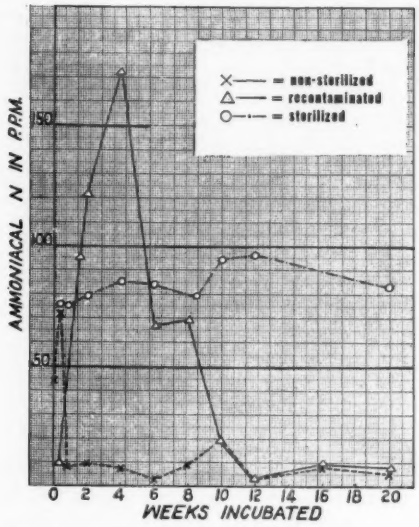


FIG. 11. *Ammonification in non-sterilized, recontaminated sterilized, and sterilized Vegreville black park soils.*

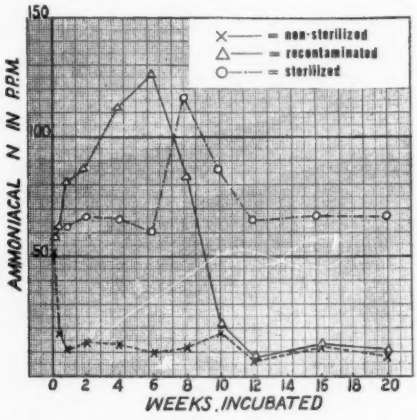


FIG. 12. *Ammonification in non-sterilized, recontaminated sterilized, and sterilized Gros Ventre brown prairie soil.*

weeks. However, in the case of recontaminated Fallis soil the accumulation of ammonia was relatively small, and it took longer to return to the normal low level. The accumulation of ammonia was roughly proportional to the

organic matter content of the soil: Edmonton, 198 p.p.m. of ammoniacal nitrogen; Vegreville, 172; Gros Ventre, 126; and Fallis, 63 p.p.m.

During the first few days of the experiment there was a temporary increase of ammonia in the unsterilized soils. This was probably the result of bringing

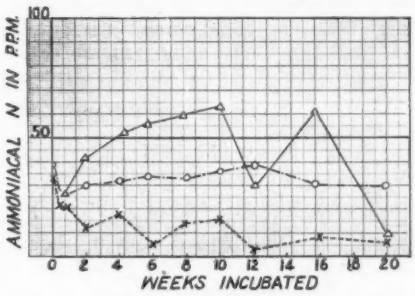


FIG. 13. *Ammonification in non-sterilized, recontaminated sterilized, and sterilized Fallis gray wooded soil; X —— X = non-sterilized; △ —— △ = recontaminated; O—·—·—O = sterilized.*

to optimum conditions of moisture and temperature soils that had been kept air-dry, thus renewing the activity of ammonifying micro-organisms. The nitrifiers apparently do not become active as quickly as the ammonifiers when air-dry soil is remoistened, but after a short period of time they convert nearly all of the available ammonia to nitrate.

The ammonia content of the sterilized soils remained at a fairly constant level above that of the unsterilized. This was probably due to the production of ammonia compounds that were absent before heating (17), but the fluctuations from this more or less constant level for each soil were probably due to contamination from the air, since each flask was opened every week or 10 days in order to add sterilized water. Great care was exercised, when watering flasks, to prevent recontamination, by flaming the mouth of each flask and keeping it open just long enough to add water. Nevertheless the cotton plug through which was placed a stirring rod for cultivating and mixing the soil after watering became loose in some flasks after repeated cultivation.

A shorter experiment on ammonification was also set up as a duplicate of the longer experiment, and ammonia was determined after three and six days, two weeks, and four weeks after sterilization. The results of this experiment are not given because the figures show the same trends as those of the first experiment.

Since the method used to determine ammonia involved the use of magnesium oxide to displace ammonia on distillation of soil extract, it was thought advisable to find out whether magnesium oxide would break down dissolved organic matter and liberate ammonia in stages rather than cause it to be given off completely in one stage. The distillations were carried out under uniform conditions for 45 min. from first boiling, during which time approximately

250 cc. of distillate were collected. When the distillation was prolonged for 45 min., a very small amount of additional ammonia nitrogen was obtained: about 4 p.p.m. as an average of 30 determinations.

For example, in one case more than 90 p.p.m. of ammoniacal nitrogen were distilled off in 10 min., 135 in 15 min., and 180 in 20 min., when the total was less than 200 p.p.m. Distilling for 45 min., from the first boiling, would therefore allow more than enough time for displacement of readily displaceable ammonia.

Sulphofication

Generally speaking there was some increase in water soluble sulphate in all four unsterilized soils on incubation, as shown in Table V, and large increases in all sterilized recontaminated soils, except Fallis. However, the sterilized

TABLE V

Sulphofication in non-sterilized, sterilized, and recontaminated sterilized Alberta soils

(Average of duplicates, p.p.m. sulphate on water-free basis)

Soil	Treatment	Period of incubation after sterilizing soil and setting up experiment				
		Right after ster.	Weeks			
			2	4	8	12
		Sulphate, p.p.m., average of duplicates				
Edmonton black park	Non-sterilized	71	64	66	69	79
	Sterilized and recontaminated	98	103	94	126	223
	Sterilized	96	114	79	124	141
Vegreville black park	Non-sterilized	76	68	70	126	101
	Sterilized and recontaminated	136	123	100	158	188
	Sterilized	122	147	85	124	152
Gros Ventre brown prairie	Non-sterilized	42	52	—	89	80
	Sterilized and recontaminated	70	68	86	105	106
	Sterilized	110	102	86	108	126
Fallis gray wooded	Non-sterilized	24	42	81	38	39
	Sterilized and recontaminated	50	42	54	57	52
	Sterilized	43	58	36	47	49

soils also showed some fluctuations and apparent increases in water soluble sulphate on incubation, and the results as a whole are not entirely consistent.

Sterilization alone considerably increased the sulphate content of each soil, as shown in the first column of Table V, but this may be due merely to an increase in soluble organic matter, which on ignition gives higher sulphate

TABLE VI

WATER SOLUBLE SULPHATES WITH AND WITHOUT IGNITION

(Average of duplicates, p.p.m. SO_4 on water-free basis)

Soil (incubated 12 weeks)	Treatment	Without ignition, SO_4 p.p.m.	With ignition, SO_4 p.p.m.
Edmonton black parkland	Non-sterilized	49	79
	Sterilized and recontaminated	185	222
	Sterilized	81	140
Vegreville black parkland	Non-sterilized	64	100
	Sterilized and recontaminated	127	187
	Sterilized	95	151
Gros Ventre brown prairie	Non-sterilized	45	79
	Sterilized and recontaminated	83	105
	Sterilized	70	125
Fallis gray wooded	Non-sterilized	18	38
	Sterilized and recontaminated	23	51
	Sterilized	15	48

results. Increases in sulphate on incubation are probably brought about by micro-organisms that oxidize proteins and other forms of organic matter that contain sulphur.

A comparison of the amounts of water soluble sulphate found with and without ignition of evaporated extracts is shown in Table VI. Without ignition only about two-thirds as much sulphate was obtained as with ignition, indicating that part of the sulphate obtained on ignition comes from soluble organic matter.

Acknowledgments

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THE FESCUE GRASSLAND OF ALBERTA[1]

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Abstract

A distinction is drawn between the virgin fescue prairie described as the *Festuca scabrella* association, and the fescue grassland produced by mowing for hay or by grazing. The virgin or climax association, dominated by *Festuca scabrella*, is characterized by some 150 species of higher plants. Various of the associated species, notably a few of the grasses, increase greatly in frequency and coverage when the prairie is used as hay meadow or as pasture, while the fescue shows a corresponding decline. Where grazing has been heavy the fescue has virtually disappeared. The fescue association is considered in relation to *Stipa*, poplar, and other contiguous plant communities. The name 'submontane' for the fescue prairie of Alberta and Saskatchewan is inappropriate. The fescue association is believed to have been associated with the formation of the black soils of Alberta, including the soils of those black and gray-black zones occupied in recent times by wooded vegetation. The bulk of the organic matter in the black soils has apparently derived from a single species of grass, viz. *Festuca scabrella*. An understanding of climax communities and successional relationships is basic to a proper classification of range land for lease purposes, and to sound long-term administrative policy for this land.

Introduction

Many parts of the vast prairie formation of North America have been carefully investigated by ecologists, but the more northern areas along the foothills of Alberta and on the southern fringe of the boreal forest in Canada have received little critical study. Weaver and Clements (9) include these northern areas in their "Mixed Prairie" association though they do not recognize *Festuca scabrella*, actually the dominant species of these areas, as one of the chief grasses of the association. Clements and Clements (3) make brief reference to a "submontane" type of mixed prairie extending from northern California and Nevada through all of nonforested Idaho and for some distance into Montana and Utah. They point out that the submontane type has its counterpart at moderate levels in central Alberta and Saskatchewan and mention the chief grasses, including *Festuca scabrella*, of this prairie type. Clarke *et al.* (1) following Clements adopt the rather inappropriate name "submontane" for the fescue grassland of the foothill and grove belt regions of western Canada. Moss (5) describes the grassland of the grove belt or parkland in Alberta as the "northern prairie" and regards *Festuca scabrella* as one of the chief dominants. For a limited area in southwestern Alberta, Moss (6) recognizes a *Festuca–Danthonia* association. The present paper presents additional data for the fescue grassland of Alberta, with a view to clarifying the ecological concepts and nomenclature involved. The paper deals also with certain practical aspects of grassland ecology, especially problems of land-use classification and the successional changes

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induced by utilization. Some consideration is given to the matter of vegetational change in response to major climatic shifts and to the role of vegetation in soil formation. For a discussion of the climate, soil zones, and topography of the region, reference may be made to earlier papers (1, 2, 5, 6).

Methods

During the past three years we have been able to study more extensively and more critically than hitherto, representative relict prairie areas in the foothill and parkland regions of Alberta. Relict areas are now exceedingly rare and soon will have become practically non-existent unless measures are taken promptly to preserve some of those that still remain. One of the best of these areas, located some 15 miles southeast of Cardston was being broken even as we were making our records. For comparison with the relict samples, which we regard as essentially virgin and climax prairie, we studied near-by grassland that had been moderately grazed or mowed for a number of years. Heavily grazed areas, by no means uncommon, were also examined. Special attention was paid to vegetational variations associated with the more obvious differences in soil and topography.

For the various areas studied the species were listed and their prevalence recorded by use of the following symbols and by supplementary notes.

Symbols shown in Tables I, II, III.

d	Dominant
a	Abundant
f	Frequent, quite common
s	Scattered, fairly common
o	Occasional, uncommon
r	Rare
vr	Very rare

Each of these symbols records an estimate of the dispersion, density, and coverage of a species.

Special attention was given to the frequency and coverage of grass species. Quantitative records for statistical treatment were not made, though the observations reflect a background of previous experience in numerical recording of vegetation cover. Certain quantitative results for utilized fescue grassland under the name "submontane mixed prairie" have been published (1).

Sample areas in each of the following regions (Fig. 1) were studied: Edmonton, Vermilion, Alix, Drumheller, Hand Hills, Calgary–Cochrane, Porcupine Hills, Pincher–Waterton, Cardston. In Table I, the first five of these regions are referred to as "north", the last four as "south". The entries in Tables I, II, and III represent a synthesis of the records for the various areas and regions. Since the sample areas of the different regions varied considerably in number, size, and suitability, and since some regions were studied more intensively than others, the results are not strictly comparable in every respect;

but they do provide a fairly accurate over-all picture of the range and relative importance of the different species in the fescue grassland.

The *Festuca scabrella* Association

This association is believed to have comprised the greater part of the native or virgin grassland found in the black soil zones of Alberta (Fig. 1). In addition to grassland these zones had large areas of poplar and other forest.

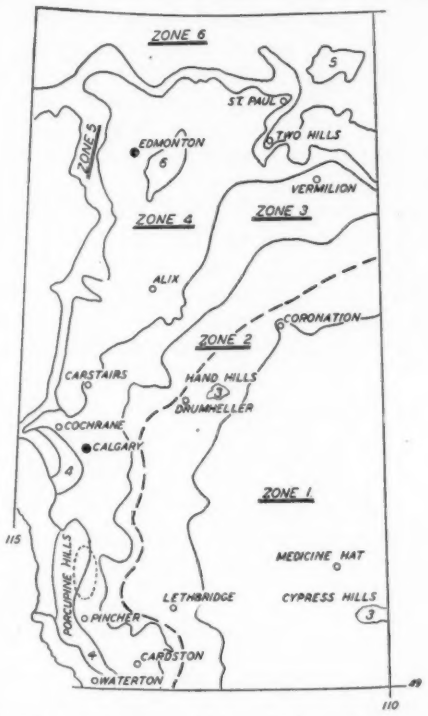

FIG. 1. *Map of central and southern Alberta showing soil zones and the approximate tension zone (heavy broken line) between climax Festuca and Stipa associations. Soil zones per Wm. Odynsky.*

Zone 1—light brown soil.
Zone 2—dark brown soil.
Zone 3—shallow black soil.
Zone 4—deep black soil.
Zone 5—gray-black (transition) soil.
Zone 6—gray (wooded) soil.

Extending approximately through the middle of the black soil region was a broad transition belt of vegetation, the grove belt or parkland, consisting of patches of prairie and aspen poplar vegetation (5). For reasons given below, the *Festuca scabrella* association is thought to have preceded forest on black soil areas recently characterized by trees. It is believed too that this association originally dominated in the transition gray-black soil zone currently

covered by forest. In the dark brown soil zone, the fescue association was intermixed with a *Stipa* association, the climax prairie of the light brown soil zone. The broken line shown on the map (Fig. 1) in the dark brown soil zone is taken to represent approximately the middle of the natural tension zone between the *Festuca* and *Stipa* associations.

In nearly all parts of the assocation, *Festuca scabrella* grows almost to the exclusion of other higher plants. This grass forms large bunches or tussocks from 1 to 3 ft. high and 10 to 20 in. in diameter. Between the tussocks the 'characteristic' associated species make relatively feeble growth. Only locally where edaphic conditions are somewhat exceptional does the fescue lose its dominance, e.g. on a gravel bank, or where a burrowing animal has disturbed the soil. The role of grazing animals in the natural biome is discussed below. While *Festuca scabrella* is undoubtedly the dominant species of the association, *Danthonia Parryi* appears to be a local dominant of considerable importance in restricted areas, especially on shallow soils of rocky and gravelly slopes. Since *D. Parryi* seems also to replace *F. scabrella* under grazing, the exact role of this grass in the virgin grassland is difficult to determine, and further investigation is needed. For the present, it seems best to regard *D. Parryi* as forming an edaphic climax within the *F. scabrella* association. The earlier proposal (6) of a *Festuca–Danthonia* association, while descriptive of typical utilized prairie of southwestern Alberta, does not denote the virgin or climax grassland of that region.

As set forth in Table I the *Festuca scabrella* association includes approximately 150 species of higher plants. There are 20 grasses, 3 sedges, 10 shrubs

TABLE I

SPECIES OF THE *Festuca scabrella* ASSOCIATION

—	Throughout the range	North only	South only	Change with grazing
Grasses				
Agropyron dasystachyum (Hook.) Scribn.	r			o–s
" *Griffithsii* Scribn. & Smith			r	o–s
" *spicatum* (Pursh) Scribn. & Smith			r	o
" *trachycaulum* (Link) Malte var. *unilaterale* (Cassidy) Malte	s			f–a
" *glaucum* (Pease & Moore) Malte				
" *typicum* Fern.	r			o
Agrostis scabra Willd.	r			o
Avena Hookeri Scribn.	r			o–s
Bromus anomalus Rupr.		vr		
Calamagrostis montanensis Scribn.	r			o–s
Danthonia intermedia Vasey	r			o–f
" *Parryi* Scribn.			o–d	o–d
Festuca idahoensis Elmer			f	s–a
" *scabrella* Torr.	d			a–f
Koeleria cristata (L.) Pers.	o			f
Poa Canbyi (Scribn.) Piper (incl. *Poa lucida* Vasey)			r	o–s
" *Cusickii* Vasey			r	o–s

TABLE I—*Continued*

SPECIES OF THE *Festuca scabrella* ASSOCIATION—*Continued*

—	Throughout the range	North only	South only	Change with grazing
Grasses—Concluded				
Poa interior Rydb.	r			o–s
Stipa columbiana Macoun			r	o–s
" *Richardsonii* Link			r	o
" *spartea* Trin.	r			r
" *spartea* var. *curtiseta* Hitchc.	o			s–f
" *viridula* Trin.	r			o–s
Sedges				
Carex Eleocharis Bailey			o	f
" *heliophila* Mack.	o			f
" *obtusata* Lilj.	r			s
Shrubs				
Amelanchier alnifolia Nutt.	r			s
Dasiphora fruticosa (L.) Rydb.			o	f
Elaeagnus argentea Pursh	r			
Prunus melanocarpa (A. Nels.) Rydb.	r			o
Ribes setosum Lindl.	r			
Rosa arkansana Porter (incl. *R. alcea* Greene & *R. suffulta* Greene)	o			s
" *Woodsii* Lindl. (incl. *R. Macounii* (Greene)	r			s
Salix Bebbiana Sarg.	s			
" " var. *perrostrata* Schneid.		vr		
Spiraea alba Du Roi				
Symphoricarpos occidentalis Hook.	s			
Forbs, etc.				
Achillea Millefolium L.	o			s
Agoseris glauca (Nutt.) Greene (incl. var. *dasycephala* (T. & G.) Jepson)	r			
Allium cernuum Roth	r			
" *textile* Nels. & Macbr.	r			
Androsace puberulenta Rydb.	r			o
Anemone cylindrica A. Gray	r			o
" *globosa* Nutt.	o			s
Antennaria aprica Greene	r			o
" *campestris* Rydb.	r			o
" *concinna* A. Nels.		vr		
" *nitida* Greene	o			f
" *pulcherrima* (Hook.) Greene			vr	o
" *rosea* (D. C. Eat.) Greene	r			s
Arabis divaricarpa A. Nels.	r			
" *hirsuta* (L.) Scop.	o			
" *Holboellii* Hornem. var. *retrofracta* (Grah.) Rollins			r	
Arnica Chamissonis Less.	r			
Artemisia camporum Rydb.	r			o

TABLE I—*Continued*

SPECIES OF THE *Festuca scabrella* ASSOCIATION—*Continued*

——	Throughout the range	North only	South only	Change with grazing
Forbs, etc.—Continued				
Artemisia frigida Willd.	o			f
" *gnaphalodes* Nutt.	o			f
Aster adsurgens Greene (*A. crassulus* Rydb.)	r			o
" *alpinus* L. (*A. culminis* A. Nels.)			r	
" *commutatus* (T. & G.) A. Gray	o			s
" *laevis* L. (incl. *A. Geyeri*)	o			s
Astragalus bisulcatus (Hook.) A. Gray			r	o
" *Drummondii* Dougl.	r			
" *flexuosus* Dougl.	r			
" *goniatus* Nutt.	o			
" *pectinatus* (Hook.) Dougl.			r	o
" *striatus* Nutt.	o			
Balsamorrhiza sagittata (Pursh) Nutt.			o	s
Campanula rotundifolia L.	r			
Castilleja ? lauta A. Nels. *lutescens* (Greenm.) Rydb. *lutea* Heller			r	
Cerastium arvense L.	o			f
Cheirinea inconspicua (S. Wats.) Rydb.	r			
Cirsium Flodmanii (Rydb.) Arth.	r			o
" *undulatum* (Nutt.) Spreng.		vr		
Comandra pallida A. DC.	r			s
Crepis intermedia A. Gray			vr	
Dodecatheon Cusickii Greene			r	
Draba nemorosa L.	r			
Drymocallis agrimonioides (Pursh) Rydb.	r			o
Erigeron caespitosus Pursh	o			f
" *formosissimus* Greene			vr	
" *glabellus* Nutt.	r			o
" " var. *pubescens* Hook.	o			s
Fragaria glauca (S. Wats.) Rydb.	r			s
Fritillaria pudica (Pursh) Spreng.			vr	
Gaillardia aristata Pursh	r			s
Galium boreale L.	o			f
Gentiana Amarella L.	r			o
Geranium viscosissimum Fisch. & Mey.			o	s
Habenaria viridis (L.) R. Br. var. *bracteata* (Muhl.) A. Gray	r			
Hedysarum alpinum L. var. *americanum* Michx. ex Pursh	r			o
var. *philoscia* (A. Nels.) Rollins				
" *Mackenzii* Richards.			o	s
" *sulphurescens* Rydb.			r	
Helianthus rigidus (Cass.) Desf. (*H. subrhomboideus* Rydb.)	vr			o
Heuchera cylindrica Dougl. var. *septentrionalis* R. B. L. (*H. Richardsonii* R. Br.)	r			o
" *flabellifolia* Rydb. var. *typica* R. B. L.			r	
Hieracium canadense Michx.	r			
Lepachys columnifera (Nutt.) Rydb.			r	o
Liatris punctata Hook.			o	s
Lilium philadelphicum L. var. *andinum* (Nutt.) Ker	r			
Linum Lewisii Pursh			r	o
Lithospermum ruderale Lehm.			o	f

TABLE I—*Concluded*

SPECIES OF THE *Festuca scabrella* ASSOCIATION—*Concluded*

—	Throughout the range	North only	South only	Change with grazing
Forbs, etc.—Concluded				
Lomatium macrocarpum (H. & A.) C. & R.			r	o
" *simplex* (Nutt.) Macbr. var. *leptophyllum* (Hook.) Mathias			r	o
" *trinernatum* (Pursh) C. & R.			r	
Lupinus argenteus Pursh			vr	o
" *leucopsis* Agardh.			o	f
Lychnis Drummondii S. Wats.	r			
Lygodesmia juncea (Pursh) D. Don.	r			
Orophaca caespitosa (Nutt.) Britt.			vr	
Orthocarpus luteus Nutt.	vr			o
Oxytropis gracilis (A. Nels.) K. Schum.	o			s
" *Macounii* (Greene) Rydb.			r	o
" *splendens* Dougl.	r			o
" *viscida* Nutt.			vr	o
Peltigera spp.	r			o
Penstemon albidus Nutt.			vr	
" *confertus* Dougl.			vr	
" *gracilis* Nutt.	vr			
" *nitidus* Dougl.			vr	o
Petalostemon purpureus (Vent.) Rydb.	r			o
Plantago septata Morris			vr	
Polygonum bistortoides Pursh			r	
" *Douglasii* Greene			vr	
Polytrichum juniperinum Willd.	r			
" *piliferum* Schreb.	r			
Potentilla gracilis Dougl.	o			
" *Hippiana* Lehm.			r	o
" *strigosa* Pall.	o			
Ranunculus cardiophyllus Hook.	r			o
" *ovalis* Raf.	r			o
Rhinanthus Kryollae Chab.			vr	
Senecio canus Hook.	r			o
" *Purshianus* Nutt.				
Senecio columbianus Greene			r	
" *integerrimus* Nutt.				
Sieversia ciliata (Pursh) G. Don.	o			f
Silene multicaulis Nutt.			vr	
Sisyrinchium angustifolium Miller	r			o
Smilacina stellata (L.) Desf.	r			o
Solidago decumbens Greene var. *oreophila* (Rydb.) Fern.	o			s
Solidago nemoralis Ait. var. *decemflora* (DC.) Fern.		r		o
" *missouriensis* Nutt. incl. *S. glaberrima* Martens	o			f
" *mollis* Bartl.			r	o
Spiranthes Romanzoffiana Cham.	vr			
Stellaria longipes Goldie	r			
" *stricta* Richards.				
Thalictrum venulosum Trel.	r			o
Thermopsis rhombifolia (Nutt.) Richards.	r			s
Vicia sparsifolia Nutt.			o	s
Viola adunca J. E. Smith	r			o
" *vallicola* A. Nels.	vr			
Zizia cordata (Walt.) Koch	r			o
Zygadenus gramineus Rydb.			o	s

and about 115 forbs. A few mosses and lichens are also recorded. Some 25 of the total number are very rare or quite localized, leaving about 125 that may be designated 'characteristic' species. Of these 125 species, some 70 may be described as 'constant', for they are likely to be found in any sizable area (several acres) of the association. In a small sample of the association, such as a few square rods, the number of these 'constants' would probably be not more than 25. These various figures could be considerably increased through more extensive investigation of the association, as also by admitting finer taxonomic divisions in certain of the species groups.

In addition to the species shown in Table I many others (Table II) have been found in fescue grassland but, for various reasons, these are excluded from the *Festuca scabrella* association. These species occur in fescue grassland that has been more or less modified through grazing or mowing, or occupy small special habitats within the prairie. They are characteristically species of other plant communities. It must be emphasized, however, that some of the decisions about the association status of species are quite arbitrary; certain species might be placed either in Table I or Table II. In passing it may be noted that the total number of species for the fescue grassland as listed in Tables I and II is approximately 260.

TABLE II

SPECIES IN THE FESCUE GRASSLAND, BUT NOT BELONGING TO THE *Festuca scabrella* ASSOCIATION

—	Moist or shaded areas	Dry or denuded areas	Change with grazing
Grasses			
Agropyron albicans Scribn. & Smith		r	o
" *Smithii* Rydb.		o	f
var. *molle* (Scribn. & Smith) Jones		s	f–a
Andropogon scoparius Michx.		o	f
Bouteloua gracilis (H.B.K.) Lag.		o	f–a
Bromus carinatus Hook. & Arn.			
var. *marginatus* (Nees) Hitchc.	o		
" *ciliatus* L.	r		
" *Pumpellianus* Scribn.	o		
Calamagrostis inexpansa A. Gray	r		
" *purpurascens* R. Br.	r		
Calamovilfa longifolia (Hook.) Scribn.		r	o
Danthonia californica Boland			
var. *americana* (Scribn.) Hitchc.	vr		
Deschampsia caespitosa (L.) Beauv.	s		
Elymus Macounii Vasey	r		
Festuca ovina L.		r	
Hierochloe odorata (L.) Wahl.	o		
Muhlenbergia cuspidata (Torr.) Rydb.		o	f
" *racemosa* (Michx.) B.S.P.	r	r	
" *Richardsonis* (Trin.) Rydb.	r	r	s
Oryzopsis canadensis (Poir.) Torr.	r		
Poa juncifolia Scribn.		r	o
" *palustris* L.	s		
" *pratensis* L.		o	f

TABLE II—*Continued*

SPECIES IN THE FESCUE GRASSLAND, BUT NOT BELONGING TO THE *Festuca scabrella* ASSOCIATION—*Continued*

——	Moist or shaded areas	Dry or denuded areas	Change with grazing
Grasses—Continued			
Poa secunda Presl.		s	f–a
Schizachne purpurascens (Torr.) Swallen	o		
Stipa comata Trin. & Rupr.		o	f–a
Sedges			
Carex filifolia Nutt.		r	s
" *praegracilis* W. Boott	r		
" *prairea* Dewey	r		
" *praticola* Rydb.	r		
" *scirpiformis* Mack.	r		
" *siccata* Dewey		r	s
" *Sprengelii* Dewey	vr		
" *xerantica* Bailey	r		
Shrubs			
Arctostaphylos uva-ursi (L.) Spreng.		r	o
Artemisia cana Pursh		r	o
Betula glandulosa Michx.	r		
" *microphylla* Bunge	r		
Juniperus horizontalis (Moench) Rydb.		r	o
Populus tremuloides Michx.	r		
Salix discolor Muhl.	r		
" *petiolaris* Smith	r		
" *pyrifolia* Anders.	r		
Forbs, etc.			
Actinea acaulis (Pursh) Spreng. (*Tetraneuris acaulis*)		vr	o
Agastache anethiodora (Nutt.) Britt.	r		
Allium Schoenoprasum L. var. *sibiricum* (L.) Hartm.	r		
Anemone canadensis L.	r		
Apocynum androsaemifolium L.		r	
Arabis Nuttallii Robinson	r		
Artemisia caudata Michx.		r	
Aster coerulescens DC.	r		
Astragalus missouriensis Nutt.		r	
Besseya wyomingensis (A. Nels.) Rydb.		r	
Bupleurum americanum Coult. & Rose		r	
Camassia esculenta Lindl.	r		
Ceratodon purpureus (L.) Brid.		o	s
Chrysopsis spp.		o	s
Cladonia spp.		o	f
Collomia linearis Nutt.	r		
Crepis runcinata (James) T. & G.	r		
Cryptantha Bradburiana Payson		vr	
Dodecatheon pauciflorum (Durand) Greene	r		
Epilobium anguslifolium L.	r		

TABLE II—*Concluded*

SPECIES IN THE FESCUE GRASSLAND, BUT NOT BELONGING TO THE *Festuca scabrella* ASSOCIATION—*Concluded*

——	Moist or shaded areas	Dry or denuded areas	Change with grazing
Forbs, etc.—Continued			
Erigeron speciosus (Lindl.) DC.	vr		
" *subtrinervis* Rydb. (*E. conspicuus* Rydb.)	vr		
Eriogonum flavum Nutt.		r	o
Gaura coccinea Pursh		r	s
Geum macrophyllum Willd. var. *perincisum* (Rydb.) Raup	r		o
" *strictum* Soland	r		o
Glycyrrhiza lepidota (Nutt.) Pursh	r		o
Grindelia perennis A. Nels.		r	o
Gutierrezia diversifolia Greene		o	f
Hedysarum boreale Nutt. var. *typicum* Rollins		r	o
" *boreale* Nutt. var. *cinerascens* (Rydb.) Rollins		r	o
Helianthus subtuberosus Bourg.	vr		
Hieracium griseum Rydb.	vr		
Hymenopappus filifolius Hook.		r	
Hymenoxys Richardsonii (Hook.) Cockerell		o	f
Juncus balticus Willd. var. *montanus* Engelm.	r		
" *Vaseyi* Engelm.	vr		
Lathyrus ochroleucus Hook.	r		
Linum rigidum Pursh		r	
Luzula campestris DC. var. *multiflorus* (Ehrh.) Celak	vr		
Malvastrum coccineum (Pursh) A. Gray		r	s
Mentha canadensis (L.) var. *glabrata* Benth.	vr		
Monarda menthaefolia Benth.	r		
Musineon divaricatum (Hook.) Raf. var. *Hookeri* (Nutt.) Mathias		r	o
Nabalus racemosus (Michx.) DC.	vr		
Orobanche fasciculata Nutt.		vr	
Oxytropis deflexa (Pall.) DC.	r		
Parnassia multiseta (Lebed.) Fern. (*P. palustris* L.)	r		
Paronychia sessiliflora Nutt.		o	s
Penstemon procerus Dougl.	r		
Perideridia Gairdneri (H. & A.) Mathias (*Carum Gairdneri*)	r		
Petalostemon candidus (Willd.) Michx.		r	o
Phlox Hoodii Richards.		o	f
Polygala Senega L.	vr		
Potentilla camporum Rydb.	r		
" *concinna* Richards.		r	o
" *viridescens* Rydb.	vr		
Selaginella densa Rydb.		o	f
Sideranthus spinulosus (Pursh) Sweet		r	o
Solidago gigantea Ait. var. *leiophylla* Fern.	r		
" *gilvocanescens* (Rydb.) Smyth (*S. canadensis* var. *gilvocanescens*)	r		
" *rigida* L. (*Oligoneuron canescens*)	r		o
Stachys scopulorum Greene	r		
Steironema ciliatum (L.) Raf.	r		
Vicia americana Muhl.	vr		
Zygadenus elegans Pursh	r		

Of considerable interest is the fact that the *Festuca scabrella* association as it occurs in southern Alberta is much richer in associated species than it is northward and eastward. As shown in Table I approximately 150 species have been recorded for the southern region and slightly over 100 species for the northern portion of the association. Some 50 species found in the south are not recorded for the north, while only four species in the north are not noted for the south. The richer flora of the southern part of the association may be explained primarily in terms of proximity to a variety of other vegetational types, especially of the mountains. Through mountain passes and also southward there is actual continuity with extensions of the Palouse prairie of the northwestern United States. Many migrant species from the west and south have apparently found niches in the fescue association of southern Alberta. Characterized by a wide diversity of edaphic conditions associated with a rough topography, this region would seem to favor the establishment of migrants. The absence of these species northward may be explained in part by the somewhat narrow habitat range for the fescue association there, but mainly in terms of the time element, insufficient time having elapsed since glaciation for these southern species to have reached the north.

As pointed out in an earlier paper (6) notable extensions of Palouse prairie species into our region are *Festuca idahoensis*, *Stipa columbiana*, *Agropyron spicatum*, *Geranium viscosissimum*, *Lithospermum ruderale*, and *Balsamorrhiza sagittata*. Of these species, some have spread farther north than others. *Festuca idahoensis*, an important species in southwestern Alberta, and known also to occur eastward in Alberta to the Cypress Hills, extends northward as far as Carstairs, about 35 miles beyond Calgary (Table III). Northward through the Porcupine Hills this fescue becomes gradually less frequent, while beyond the Hills to Calgary and Carstairs it passes from the role of a scattered species to one of rare occurrence in the *Festuca scabrella* association. *Agropyron spicatum* while found chiefly on dry slopes and shallow soils of ridges in the southwestern region occurs sporadically eastward to the Cypress Hills, including stations near Del Bonito, southeast of Cardston. *Balsamorrhiza sagittata* seems not to extend north of the Porcupine Hills. *Geranium viscosissimum* and *Lithospermum ruderale* are found at least as far north as Carstairs. Other species of considerable importance in the southern part of the association, but not recorded for the north include: *Lupinus leucopsis*, *Liatris punctata*, *Vicia sparsifolia*, *Zygadenus gramineus*, and *Dasiphora fruticosa*. The last species, the shrubby cinquefoil, is another of the southern species with an extension eastward in the Cypress Hills and also northward a considerable distance—approximately 70 miles north of Calgary.

Danthonia Parryi is worthy of a special note. This grass with a range from Alberta to New Mexico seems to have received little attention from ecologists. While apparently unimportant as a range plant in the United States it is a grass of some consequence from Waterton northward through the Porcupine Hills. In the northern part of the Porcupine Hills and beyond Calgary, this

TABLE III

GRASSES AND SEDGES IN APPROXIMATE ORDER OF THEIR IMPORTANCE IN THE FESCUE ASSOCIATION, SHOWING THEIR GEOGRAPHICAL OCCURRENCE AND THE CHANGES IN FREQUENCY AND COVER PRODUCED BY MODERATE GRAZING AND MOWING (SEE FIG. 2)

	A	B	C	D	E	F	G	H	I	*
Festuca scabrella	d	d	d	d	d	d	f–d	f–d	f–d	a–s
" *idahoensis*						o	o–f	f	f	s–a
Agropyron trachycaulum	s	s	s	s	s	s	s	s	s	f–a
Koeleria cristata	o	o	o	o	o	o	o	o	o	f
Stipa spartea var. *curtiseta*	r	o	o	o	o	o	o	o	o	s–f
Danthonia intermedia	r	r	r	r	r	r	r	r	r	o–f
" *Parryi*						o	s	f	f	o–d
Avena Hookeri	r	r	r	r	r	r	r	r	r	o–s
Poa interior	r	r	r	r	r	r	r	r	r	o–s
" *Canbyi*			r	r	r		r	r	r	o–s
" *Cusickii*						r	r	r	r	o–s
Agropyron dasystachyum	r	r	r	r	r	r	r	r	r	o–s
" *Griffithsii*					r	r	r	r	r	o–s
" *spicatum*							r	r	r	o
Stipa viridula	r	r	r	r	r	r	r		r	o–s
" *columbiana*						r	r	r		o–s
" *Richardsonii*						r	r	r		o
" *spartea*			r			r	r			r
Calamagrostis montanensis		r	r	r	r	r	r	r	r	o
Agrostis scabra	r	r	r		r	r	r	r	r	o
Bromus anomalus	r		r	r	r	r				r
Carex heliophila	o	r	r	o	r	r	o	o	o	f
" *Eleocharis*		r			r	r	o	o	o	f
" *obtusata*	r	r	r	r	r	r	r	r	r	s

A—Edmonton *D—Drumheller* *G—Porcupine Hills*
B—Vermilion *E—Hand Hills* *H—Pincher–Waterton*
C—Alix *F—Calgary–Cochrane* *I—Cardston*

**—Changes produced by moderate grazing and mowing.*

species decreases in frequency. North of Calgary it occurs sporadically as far as the Carstairs region where it becomes rare (Table III).

Previous accounts (1, 5, 6) of the vegetation of our region, while recognizing the importance of *Festuca scabrella*, gave a large place also to certain other grasses, notably, *Agropyron* spp., *Stipa* spp., *Koeleria cristata*, *Poa* spp., *Danthonia* spp., *Festuca idahoensis*, and *Avena Hookeri*, as well as to certain sedges and forbs. These descriptions actually refer to grassland that has been considerably modified through grazing or mowing, and not to the virgin or climax state. In these earlier publications, as well as in many papers on grassland elsewhere, there is no clear indication as to whether the recognized communities are climax or disclimax. As shown in Table I and Fig. 2, the secondary grasses and sedges have a relatively small place in the climax community but become leading species in the modified grassland.

The words 'virgin' and 'climax' used synonymously above in reference to the *Festuca scabrella* association, demand some clarification. 'Virgin' is used in the usual sense of the prairie as white man first found it. 'Climax' has

reference to the grassland community that had developed, or eventually would develop, under climatic conditions prevailing in the region. Both terms take into account the whole biota, including aboriginal man and native grazing animals, and their influence on the grassland. The role of burning as employed by Indian tribes and the effect of bison grazing will be discussed below, but here the view may be expressed that these factors did not markedly disturb the 'virgin' or 'climax' fescue association that had developed in much of the region.

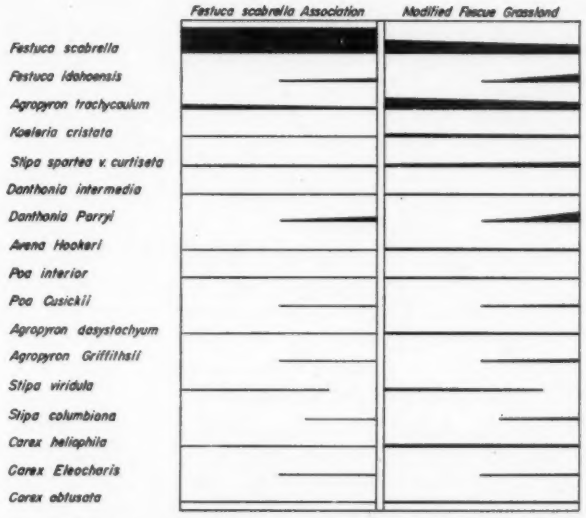

FIG. 2. *Graphical representation of the leading grasses and sedges in the Festuca association of Alberta, showing the relative abundance of the species from north to south, and the changes produced in this grassland by mowing and by moderate grazing (see Table III).*

Modified Fescue Association

The *Festuca scabrella* association exists in concrete form today only as rare relict areas. Practically all of this association, the virgin fescue grassland, has been either broken or greatly altered through mowing or grazing. Though the unbroken land is often referred to as native or natural prairie, most of it has a plant cover very different from the original. Some of the more important of these changes in the vegetation have been indicated above. They will now be discussed more fully, making reference to the tables and Fig. 2.

Through mowing of the grassland, *Festuca scabrella* has become much smaller in stature and in diameter of tussock but has remained fairly uniformly distributed. Between the tussocks, various of the 'characteristic' species have become prominent, and certain species from other communities of the region have established themselves locally. The more marked changes are seen on the poorer sites, such as stony soil and dry slopes. In these situations,

Festuca scabrella may even disappear while other species become abundant, notably *Festuca idahoensis*, *Stipa spartea* var. *curtiseta*, *Agropyron dasystachyum*, *Koeleria cristata*, *Carex* spp., and certain forbs. *Danthonia Parryi* is dominant in localized areas especially on exposed, wind-blown slopes north of Waterton Lakes Park and in the southern part of the Porcupine Hills. This grass should receive further study with a view to determining its role as an edaphic subclimax species, and its occurrence as a grazing or mowing disclimax.

Standard mowing practice for fescue prairie in Alberta is to take a crop of hay in alternate years. Occasionally, two years intervene. When mowed in alternate years a good area will yield about one ton per acre in the year taken. The meadow shown in the photograph (Fig. 3) located northwest of Calgary, had been mowed over a period of 23 years, and had previously been grazed. In 1944 when the photograph was taken, this meadow had not been cut for two years and was yielding a crop of one and a half to two tons per acre. While *Festuca scabrella* was still the chief grass over most of this meadow, various of the other grasses and also many forbs were prominent, with *Danthonia Parryi* locally dominant on some of the slopes and *Stipa spartea* var. *curtiseta* abundant on most of the drier exposed areas.

The effect of grazing of *Festuca scabrella* is somewhat different from that of mowing, in that this grass becomes quite patchy, tending to persist on the more moist situations and where given some protection from grazing animals by shrubby cinquefoil, roses, and other small woody plants. On the better sites, *F. scabrella* is often succeeded to a marked degree by *Agropyron trachycaulum* var. *unilaterale*, while *Koeleria cristata*, *Avena Hookeri*, *Stipa spartea* var. *curtiseta*, and *Danthonia intermedia* may become fairly common. On drier sites, *Agropyron trachycaulum* is likely to become less abundant than *Koeleria*, *Stipa* spp., *Festuca idahoensis*, *Danthonia Parryi*, *Agropyron dasystachyum*, *A. Griffithsii*, and *Poa* spp. The sedges increase markedly with grazing, *Carex heliophila* being found throughout, and *C. Eleocharis* often common in the south (Fig. 2).

Many of the shrubs, forbs, and other species also increase greatly under utilization (Tables I and II). While, in general, the same species tend to become prominent, whether an area is used for hay or for pasture, the shrubs and certain of the forbs, e.g. *Lupinus leucopsis* and *Artemisia* spp., are kept down by mowing and conversely become abundant under grazing.

Heavy or indiscriminate grazing brings about still more marked changes along the lines indicated for moderate grazing. *Festuca scabrella* is soon greatly reduced, or even eliminated, while various of the other grasses, sedges, and other species of the association increase. In some areas, *Carex Eleocharis* has become the chief plant. In other areas, there remains only a sparse growth of *Carex* spp., *Artemisia frigida*, *Antennaria* spp. This condition prevails in the overgrazed pasture shown in Figs. 3 and 4, an area adjoining a fescue meadow and tenanted originally by the *Festuca scabrella* association. Moreover, other species may come in, notably *Stipa comata*, *Bouteloua*

PLATE I

FIG. 3. *Fescue hay meadow near Calgary, showing haying operations. Overgrazed pasture in foreground was formerly fescue prairie.*

FIG. 4. *Same meadow and pasture as in Fig. 3, showing Festuca scabrella in the meadow, and Artemisia frigida in the pasture.*

gracilis, and *Agropyron Smithii*, species of the plant association that characterizes the light brown soil zone. Indeed these species are so common in parts of the general region as to leave the impression that they belong there. No doubt these species did occur in the fescue or black soil region under natural conditions, but only on very dry, south-facing slopes or certain kinds of clay flats. From these localized areas they have spread to overgrazed pastures. On the east side of the Porcupine Hills, for instance, *Bouteloua gracilis* has become a dominant plant in many areas originally covered by fescue. This remarkable behavior of blue grama grass should be further investigated. Also worthy of mention in this connection is *Andropogon scoparius*, which has a sporadic distribution from Calgary southward to Pincher, occurring chiefly on denuded slopes with poor soil and scant vegetation. No doubt this grass, like *Bouteloua*, has occupied certain localized areas within the fescue region for many centuries, but recently has spread somewhat to ground formerly occupied by the fescue association.

The influence of bison and other native grazing animals on the virgin prairie has been the subject of much speculation. Though *Festuca scabrella* has been called buffalo bunch grass because the buffalo (bison) were allegedly fond of it, there seems to be no evidence that this grass was excessively grazed by these animals. It is doubtful whether our fescue grassland was grazed by buffalo even to the extent now utilized by domestic animals on the best ranges of the region. Larson (4) contends that buffalo had much the same effect upon the plains grassland as is currently exerted by domestic grazing animals, which was to produce a short grass type of vegetation. Therefore, according to Larson, the short grass plain is not a "disclimax". Our studies point to the conclusion that the *Festuca scabrella* association is not only the climax grassland for our region but that this climax was, in the main, realized and maintained during the time when buffalo were transient members of the same biotic community. Had the fescue association been held in a "disclimax" condition by the buffalo, it would be difficult to account for the present distribution of the climax association. Historical records point to the conclusion that the plains buffalo did not range northward in large numbers as far as our region and that the wood buffalo remained farther north.

Fescue Prairie and Other Vegetation

The relationship between the fescue association and the grassland of southeastern Alberta has been discussed briefly above. The grassland of the southeastern region has been variously named and described in earlier publications (1, 2, 5, 6). Suffice it to say here that we prefer to characterize this prairie in terms of the climax state rather than in terms of the prevailing condition, which we are inclined to believe is largely disclimax. Since *Stipa comata* is considered to be the climax species, the climax prairie may be called the *Stipa comata* association. Important grasses in the disclimax, as it occurs today in most parts of the region, are *Bouteloua gracilis*, *Koeleria cristata*, *Poa secunda*, and *Agropyron Smithii*. The latter species is also important as a

subclimax dominant, especially on clay flats. The broken line in Fig. 1 is believed to represent approximately the middle of the tension zone between the climax *Festuca* and *Stipa* associations. Through grazing and other disturbances associated with settlement of the country, this natural tension line has been shifted considerably to the north and west. There are, of course, outliers of each association in the general region of the other, for example, the fescue association in the Cypress Hills.

The question of equilibrium between prairie and associated forest is one of perennial interest. We have little to add to the earlier discussions (3, 5, 6) of this topic for our region, except to emphasize the fact that poplar, willow, and certain other woody plants are tending to invade the fescue prairie. Regardless of the effects of earlier climatic shifts, the trend at present, which seems also to have been the main trend for many decades or even centuries, is for the woody species to encroach upon the grassland. Counteracting this tendency are the effects of burning, mowing, and, to a limited degree, grazing. In many of the ranching areas, for example, the north Porcupine Hills, willows are a constant source of annoyance in the fescue hay meadows and are kept down only by regular mowing. Similar areas, where grazed rather than mowed, have large clumps of willow (chiefly *Salix Bebbiana*), as well as other woody plants. Grazing does actually retard the development of willows and poplars, the degree of damage depending on grazing intensity, browsing habits of the animals, and the local environmental conditions. Certain other woody species in the fescue grassland, notably the shrubby cinquefoil, seem to be definitely favored by grazing. Further investigation is needed to fully elucidate the ecology of woody plants in fescue prairie under range conditions. Incidentally, we are prepared to support the opinion expressed by Roe (7) and by Soper (8) that buffalo did relatively little damage to woodland growth. As Roe emphasizes, fire seems to have been much more effective than the buffalo in causing deforestation and in retarding the advance of trees and shrubs into the prairie.

Natural succession from fescue prairie to wooded vegetation was most clearly evident in the north Porcupine Hills and north of these Hills. Here the grassland is commonly invaded by willow followed by poplar or, through root-suckering, directly by poplar. Willow and poplar are followed in due course by white spruce (*Picea glauca*), or, at higher altitudes, by *Pinus contorta* var. *latifolia* and *Pseudotsuga Douglasii*. As suggested in an earlier paper (5), white spruce may be regarded as the theoretical climax for much of the fescue grassland or black soil region, though natural burning tends to prevent the realization of this climax.

The occurrence of isolated aspen poplar groves on moist slopes throughout our parkland region has not been satisfactorily explained (5). These groves may possibly be remnants of a continuous forest cover that prevailed during a cooler wetter period. On the other hand, it seems more likely that they started individually from seed, either long ago or in relatively recent times. Critical soil studies should shed some light on this problem.

Prairie patches or 'islands' in the wooded parts of our region are readily explained as relicts of a continuous grassland that covered the region during a former drier period. The 'islands' are found on dry slopes and on light sandy soils. One of these was observed near Beauvallon, east of Two Hills, where most of the natural vegetation for many miles around was wooded. This was a level area, with light soil and a covering of grasses, sedges, and forbs characteristic of the fescue association. Though only scattered wisps of *Festuca scabrella* were found, we are confident that this grass was abundant in the natural cover, and has been reduced by rather heavy pasturing of the area. Prairie patches on very dry slopes within the wooded region are usually somewhat denuded by overgrazing. Their characteristic flora of *Stipa* spp., *Bouteloua gracilis*, *Agropyron* spp., *Carex* spp., etc., resembles the *Stipa* association more closely than the *Festuca scabrella* association. Though the *Stipa* community has no doubt been favored on these dry slopes since the advent of white man, this type of vegetation was surely established there from early times and probably since a much drier climate prevailed in the region. This aspect of the vegetation therefore lends strong support to the view that our present climate was preceded by a long drier period.

Mention may be made of a few minor aspects of the fescue grassland in relation to other types of vegetation. In many localized areas, colonies (societies) of *Symphoricarpos occidentalis* occur in the prairie. These may have developed because of special edaphic conditions or because of disturbances by animals (5). Elsewhere there are thickets of *Rosa* spp. In parts of the foothills, various other shrubs, e.g. *Betula glandulosa*, *Juniperus horizontalis*, *Arctostaphylos uva-ursi*, tend to invade the fescue grassland. On lower ground, the fescue meets other shrubs, such as *Salix petiolaris* and *S. discolor*, and also lowland grasses, sedges, rushes, etc., including such grasses as *Deschampsia caespitosa*, *Calamagrostis canadensis*, and *Poa palustris*. Where the various woody and lowland communities meet the fescue grassland, the latter is, of course, not developed as the genuine fescue community but has an admixture of foreign species. Most of the species listed for the fescue grassland in the first column of Table II belong in this category.

Grassland Vegetation and Soils

Our studies support views generally held by students of climate–soil–vegetation relationships in the steppe–woodland regions of Russia and North America, viz.

1. That black (chernozem) soils are essentially grassland soils developed through many centuries in a cool continental climate of moderate rainfall.
2. That the steppe or prairie areas, likewise the black soil areas, were originally larger than now, and have been invaded by forest rather recently in soil history.
3. That the black soils thus covered by forest have been modified by podsolization to become 'degraded chernozems' or transition (gray-black) soils.

Emphasizing here our conclusion that the natural vegetation of the black soil and gray-black soil zones through many centuries was a *Festuca scabrella* association in which a single species predominated, and assuming that most of the organic matter in prairie soils has derived directly or indirectly from the roots and crowns of grasses, we may conclude that the bulk of the organic substance in the black soils was produced by the dominant grass species, *Festuca scabrella*. Even in the dark brown soil zone, this grass seems to have flourished to some extent and no doubt has furnished that soil with much organic material. It appears therefore that a large proportion of the agricultural wealth represented by the black and related soils of our region had its origin in the activity of just one kind of higher plant.

Utilization Problems

Though this investigation emphasizes the profound changes brought about in the fescue grassland through grazing and mowing, the ecology of the dominant grass, *Festuca scabrella*, is still not well understood. The relatively high crowns of this species seem to require for their vigorous growth the shelter of old leaves and the protection mutually afforded one another. Not only grazing and mowing, but also burning, may bring about desiccation of the fescue tussocks. The role of prairie fires in relation to utilization should be further investigated.

Questions regarding the regeneration of *Festuca scabrella* remain unanswered, for instance, (*a*) the time required for recovery of fescue grassland when given a rest period and following various stages of depletion, (*b*) factors affecting seed-setting and establishment of seedlings. The heading out of this grass seems to vary greatly from place to place and from season to season, but no correlation between heading and such variables as soil, exposure, composition of stand, or even vigor of plants has been established. It may be significant that the generally good heading of this species in western and central Alberta during the year 1947 followed a winter of exceptionally heavy snowfall.

There is also the question whether, in the interest of range stability, the dominance of the relatively high forage-producing and palatable *Festuca scabrella* should be maintained, or alternatively, whether this grass might well be replaced in large part by such species as *Agropyron trachycaulum*, *Stipa* spp., *Koeleria cristata*, *Danthonia Parryi*, and certain forbs. In cattle-raising regions the areas dominated by *Festuca scabrella* are prized as native hay lands. On the other hand, in certain sheep-ranching regions the disappearance of *Festuca scabrella* is looked upon with favor as the sheep seem to prefer the fine-leaved shorter grasses. In this connection, additional information is needed on the relative nutritive values of the secondary species and on their ability to withstand grazing. Further research should be done too on the successional changes induced by utilization and on the effects of various kinds of grassland cover on soil erosion initiated by water and wind.

Though much remains to be learned concerning the proper utilization of Alberta grasslands, investigations already carried out have provided valuable

information of a practical nature (1, 2). Mention may be made of the use of quantitative studies on ground cover to establish an index as to forage yield. From this information and a knowledge of succession phenomena, grazing lease rentals have been set with some degree of success.

Acknowledgments

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AEROBIC BACTERIA THAT DECOMPOSE CELLULOSE, ISOLATED FROM QUEBEC SOILS

I. ISOLATION AND DESCRIPTION OF THE SPECIES[1]

By A. M. Alarie[2] and P. H. H. Gray[3]

Abstract

Soil bacteria that hydrolyze starch were isolated by means of an elective culture medium with this polysaccharide as the sole source of carbon; their ability to decompose cellulose was then tested. Thirteen strains of cellulose decomposing bacteria were thus isolated from several Quebec soils. The bacteria have been assigned to three recognized genera under eight new specific names. Their elevation to species rank is based on differentiations comparable to those used in Bergey's Manual. The following new species have been proposed: *Vibrio perimastix, Vibrio hyperion, Bacterium infirme, Bacillus vagans, Bacillus Kellermani, Bacillus soli, Bacillus torquens,* and *Bacillus effluens.*

Introduction

Under natural conditions cellulose is biochemically one of the most important of the compounds that reach the soil as a component of plant residues. This is especially true of forest soils and pastures. In agricultural soils cellulose is added, along with other materials of significance in microbial nutrition, by the plowing-in of the roots of many annual crops, and by the addition of farmyard manure. In soils under crops the normal equilibrium between groups of micro-organisms is disturbed by the introduction of plant residues.

Recent work on the cellulolytic (cellulose decomposing) bacteria of soil has been reviewed by Norman and Fuller (20, pp. 239-264). These authors consider that the aerobic cellulose bacteria in soil are mostly versatile organisms that, individually, may not be very vigorous in attacking cellulose but that are likely to be able to maintain themselves in a heterogeneous soil population that is normally utilizing not cellulose alone in the pure state but a substrate of which cellulose is only one of many constituents. These authors developed cellulose–dextrin media that they claim favor the growth of cellulose decomposing bacteria of the genus *Sporocytophaga* (*Myxobacteriales*) as well as that of the versatile species. Since the majority of the versatile species of cellulolytic bacteria are also recorded as capable of using starch, and since this carbohydrate is less refractory to attack by micro-organisms, it was decided to use starch instead of cellulose as the source of carbon in a mineral-salts enrichment medium in an attempt to isolate cellulolytic bacteria from

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a variety of Quebec soils. The main reason for this departure from the usual procedure of inoculating soil into media containing filter paper was the difficulty that in the latter media growth of cellulolytic bacteria was usually slow and led to the development of many associated bacteria that interfered with an easy isolation of the organisms sought for.

Summary of Recent Studies

By far the most important group of cellulose decomposing bacteria is represented by versatile organisms. They attack a wide range of carbon and nitrogen sources, though not necessarily vigorously. In a rapid survey of the literature on these bacteria we have eliminated reference to those that did not grow on starch as this carbohydrate is the basic compound of the enrichment and isolation media used in the present study. A nearly complete review of the literature, up to 1946, has been presented by Harmsen (12).

Vibrio species have been described by Gray and Chambers (8), Dubos (3), Kalnins (15), Itano and Arakawa (13), Stapp and Bortels (26), and Gray (7). Eighteen species in all showed diastatic activity. Five strains not named by Jensen (14) showed a similar aptitude.

Of the large group of *Cellulomonas* (26 species) that compose the studies of Rullmann (21), Kellerman and McBeth (16), Kellerman, McBeth, Scales, and Smith (17), McBeth and Scales (19), McBeth (18), Sack (22), and Sanborn and Hamilton (23), only one species, *Cellulomonas acidula* Kellerman *et al.* (17), did not grow on starch. Those of Rullmann and of Sack have not been fully described as regards their diastatic activity. The only *Cellulomonas* isolated from Quebec soils, by Hamilton (11), has not been thoroughly studied and cannot be catalogued here as no species name has been given.

Of the 10 species of *Bacillus* that have been reported in the studies of Kellerman and McBeth (16), McBeth and Scales (19), McBeth (18), Kalnins (15), Simola (24), Zarembska (27), and Fuller and Norman (4), one is given as not hydrolyzing starch, *Bacillus imminutus* McBeth (18). Two of Sack (22) are not sufficiently described. Jensen (14) also quoted two unidentified species as hydrolyzing starch.

The situation is similar with regard to the *Pseudomonas* group. Of the three described by Kellerman *et al.* (17), McBeth and Scales (19), and McBeth (18) and classified as *Pseudomonas* by Bergey *et al.*, 5th edition (2), and of the three given by Fuller and Norman (4), only the one of the latter authors, *P. erythra*, does not grow on starch, and seems to be an obligate cellulose and cellulose–dextrin decomposer.

Three species of unidentified *Corynebacterium* (Jensen, 14) are also starch hydrolyzers.

One species of what appears to be a *Vibrio* and called *Cytobacter polonicum* by Gutgisser (10) grew well on starch but the starch hydrolysis is not mentioned. The same applies to *Cellulococcus albus* as reported by Sack (22).

The three strains of *Bacterium cellaresolvens* α, β, and γ of Groenewege (9) and Jensen's (14) unidentified *Bacterium R* and *CO* are starch hydrolyzers. The same applies to *Achromobacter picrum* recently described by Fuller and Norman (4).

It should be noted that Bergey's Manual, 5th edition (2), reports under the genus *Cellulomonas* a number of species originally described as *Bacillus* or *Bacterium;* it should also be noted that many organisms mentioned above are not to be found in the Manual.

This concludes a general review of the versatile soil bacteria reported to decompose cellulose. As to rapidity of action, the literature suggests that the many versatile bacteria are not less active than the other forms. Both groups are widely distributed in soil and have been reported throughout the world. The diastatic power is a property of the majority of the versatile organisms above, and with this in mind, the authors have undertaken the present study.

Cellulose decomposing bacteria have been reported only on very rare occasions from Quebec soils. Hamilton (11) has described an organism active in cellulose decomposition but did not go further than the genus for the purpose of his study; the organism was a *Cellulomonas*, showing only few differences from *Pseudomonas subcreta* McBeth and Scales (19). (Bergey *et al.*, 1923, 1st edition, emended the name to *Cellulomonas subcreta*, but gives *Pseudomonas* in the 5th edition, 1939). Gray (6) investigated the microflora of separate horizons of podsol soils; he ascertained the presence of *Cytophaga Hutchinsonii* (now *Sporocytophaga myxococcoides* Stanier), and of other forms not isolated. In a more recent study Gray (7) described *Vibrio amylocella* that had been isolated from a manured garden soil.

Experimental

Methods

Soils and Methods of Sampling

Thirteen soil samples were taken at different places on the farm at Macdonald College, and from soils in different localities in the adjacent counties of Soulange and Vaudreuil, with no particular regard to the soil type or crops.

To prepare the profile for sampling, the trowel or shovel was cleaned with paper and rags, washed, and flamed with alcohol at each new profile. Samples were generally taken at from three to five inches from the top of the profile and special mention is made when this was not the case. One sample was taken of each profile. Samples were collected in small glass vials (15 × 75 mm.) previously plugged with cotton and sterilized. At each soil profile, the requisite number of vials were forced horizontally, with a slight screwing motion, into undercut and well scraped profiles in order to avoid contamination from surface dust. The bottles were then closed with sterile rubber stoppers, wrapped, and taken to the laboratory.

Isolation of Starch and Cellulose Hydrolyzing Bacteria

In order to obtain a dense growth of powerful starch hydrolyzing organisms, elective cultures were set up by inoculating 50 ml. of a starch* solution in 250 ml. Erlenmeyer flasks with approximately 1 gm. of fresh soil. The medium contained starch 0.2%, ammonium chloride 0.2%. The mineral salt solution used throughout the course of the work was composed as follows (Kalnins, 15):

	%
Potassium monohydrogen phosphate	0.1
Magnesium sulphate	0.02
Sodium chloride	0.01
Calcium chloride	0.01
Ferric chloride	0.002

After 24 hr. of growth at 28° C., the cultures were plated with starch agar, containing peptone instead of ammonium chloride, with the same mineral salts. The dilutions were made with a 3 mm. loop, transferring a loopful of the elective culture to a tube of melted agar and again a loopful from the latter to a second tube. Three such dilutions were made and that in the last tube in duplicate. The plates prepared from the tubes were incubated at 28° C. After three to five days, those colonies that seemed different were transferred to grid plates of starch agar. The underside of a starch agar plate was divided by means of wax-pencil marks into 16 squares of even dimensions and marked for orientation. A sheet of paper marked in the same fashion and numbered from 1 to 16, placed under the plate, permitted the localization of the different inoculations without turning the plate upside down at each inoculation. This arrangement insured a rapid means of identification of the different colonies. The grid plates were incubated for 48 hr. at 28° C. and then all the colonies that developed were transferred to slants of starch agar of the same composition as that in the plates. After making sure that all the organisms had grown on the slants the grid plates were flooded with an iodine solution and the organisms that hydrolyzed the starch were kept for tests on cellulose. Most of the organisms isolated in this manner were pure cultures.

The ability of these bacteria to decompose cellulose was ascertained in a medium containing filter paper as described below. A comparison of the nutrient value of different sources of nitrogen for these bacteria forms the subject matter of another paper.

Culture Media and Cultural Methods

Since many of the strains isolated proved to grow very poorly in the presence of meat extract or of nitrate salts, the culture media used throughout the tests were supplied with nitrogen in the form of peptone. The basic mineral salt solution for most of the media was that used by Kalnins (15). The different salts were kept in separate stock solutions at 100-fold concentrations. A brief description of the different culture media used is given below.

* *This was BDH AnalaR. It was incorporated into media in the manner described by the junior author (7).*

Starch Broth and Agar

Starch was mixed with 25 to 30 ml. of cold distilled water, and then poured into boiling water to dissolve. The solution was boiled for approximately one minute or until opalescent; 0.2% peptone and the mineral salt solution were then added and the medium brought to final volume.

In the case of starch agar, 1.5% of agar was added to the starch broth.

Cellulose Salts Liquid Medium

The nutrient solution was composed of 0.2% peptone and the required quantity of mineral salts in distilled water. The bacteria were cultured in tubes containing 10 ml. quantities with a strip of filter paper (Whatman No. 41) of such a length as to emerge over the surface of the liquid.

Cellulose Agar

This culture medium consisted of finely precipitated cellulose incorporated into a peptone mineral salt agar.

Six grams of filter paper were precipitated with sulphuric acid, according to the method given by Kalnins (15). Finely cut filter paper was dissolved for 20 sec. in slightly diluted sulphuric acid (100 ml. of concentrated sulphuric acid, sp. gr. 1.81, in 60 ml. of distilled water). Cellulose was reprecipitated in bringing the volume rapidly up to two litres with water and allowed to settle overnight in 10 to 15 litres of water. The supernatant liquid was siphoned off and the precipitate filtered in a large Buchner funnel with repeated washing until all traces of acid were removed. The cellulose mat obtained on filtration was resuspended in 800 ml. of water for storage; a very even precipitate was obtained by agitation with an egg beater. This gave an approximate concentration of 0.4% cellulose, which proved to be a convenient density of fibres in the agar plates.

The agar concentration was lowered to 0.8% as advised for the studies of the *Cytophaga* group (Stapp and Bortels, 26, Stanier, 25); it proved to be very valuable in these studies as it gave better evidence of enzymic action and larger colonies.

Gelatin

Two formulae for gelatin were used. The common nutrient gelatin, with 120 gm. of gelatin, was first used, but as some strains were inhibited by the presence of meat extract, it was decided to test for gelatin liquefaction in a medium containing gelatin 10%, starch 0.2%, peptone 0.2%, and the mineral salt solution.

Litmus Milk

The dehydrated medium of the Difco Laboratories were used.

Nutrient Broth and Agar

The ordinary nutrient broth was composed of meat extract 0.3%, and peptone 0.5%. Agar 1.5% was added to nutrient broth in order to obtain the nutrient agar.

Dextrose Agar

This culture medium was composed of dextrose 0.5%, peptone 0.2%, and the mineral salt solution.

Carbohydrate Broths

The different mono- and polysaccharides, alcohols, and glucosides as listed in Table I were added as 0.5% to the basal mineral salt solution with peptone 0.2%. Phenol red in 0.024% concentration was used as indicator.

Sterilization under pressure in the autoclave caused very little change in the pH, and it was assumed that the more sensitive sugars, such as glucose, were not hydrolyzed in presence of the mineral salts at a pH value of about 8.0.

Medium for Ammonia Production

The test was carried out on two-month-old cultures in cellulose solution, and on three- and six-day-old cultures in urea broth containing urea 2% and 'yeastrel' 0.3%.

Medium for Nitrate Reduction

Nitrite formation from nitrate was tested in three- and six-day-old cultures in starch mineral salt broth containing sodium nitrate 0.2%.

Medium for H_2S Production

Kligler iron agar produced by the Difco Laboratories was used to test for the production of H_2S.

Medium for Indol Production

The culture medium used for this was a Difco product, Bacto-troptone. The test for indol was made in six-day-old cultures by means of Ehrlich's reagent; 0.5 ml. of the reagent was run into slanting tubes of the culture so that it remained on top of the liquid.

Relation to Free Oxygen

The anaerobic faculty of the strains under observation was studied in 300 ml. bottles with 30 ml. of pyrogallol dissolved in 20% potassium hydroxide. A tube of reduced methylene blue was placed with the cultures in each bottle and the bottles were tightly closed with rubber stoppers. After eight days' incubation at 28° C., the bottles were opened for observation and all the tubes with methylene blue were still colorless. After exposure to atmospheric oxygen they all turned blue within a few minutes.

Staining Methods

Cell Morphology

Form and dimensions of the cells were studied on 48-hr.-old (occasionally 24 hr.) starch agar slants. The films were fixed by flame and stained with aqueous crystal violet.

Spores and Spore Staining

Spore location, form, and dimensions were determined with starch agar cultures of different ages because of the different ability of the strains to form

spores more or less rapidly. One strain, *Bacillus soli*, had to be studied on nutrient agar because it produced only a few spores on starch agar.

The Ziehl–Neelsen method for acid-fast organisms was used throughout for staining spores as it affords a counterstain, methylene blue, for the cell itself. Pretreatment for 30 sec. with chromic acid, 0.5%, proved to increase the receptivity of the stain by the spores.

Gram Stain

Hucker's modification of Gram's method was used with Atkins' sodium hydroxide – iodine solution. The stain was used with 24-hr.-old cultures on starch agar slants and repeated after eight days of growth. A 24-hr.-old culture of *B. subtilis* was used as a control.

Flagella Stain

The bacteria from a 24-hr.-old culture on starch agar slant were allowed to swarm in weak suspension, in a few drops of distilled water, for 15 min. A loopful of the suspension was then placed on a well roasted and cooled slide; from this loopful a smaller one was transferred to an adjacent loopful of distilled water. By this means many films were prepared at once on one slide. Gray's (5) method of staining flagella was used. Conventional terminology has been adopted to indicate the apparent placement of these appendages.

Results

Starch Hydrolyzing Bacteria

One hundred and three strains were isolated. Many of the organisms were alike on the basis of their colony formation and morphology.

Most of the cultures could be assigned to the genus *Bacillus*, some to *Bacterium*. A few *Pseudomonas*, two strains of *Proactinomyces*, and one *Micrococcus* were also isolated.

Only 13 strains proved to be cellulose hydrolyzers as well. These only were studied more in detail.

Cellulose and Starch Hydrolyzing Bacteria

Six only of the 13 soils examined yielded 13 strains of the bacteria, which are numbered and described below under their specific names.

The eight species are assigned to three genera, namely, five *Bacillus*, two *Vibrio*, and one *Bacterium*. The latter genus has been adopted in preference to *Cellulomonas* as advocated by Bergey *et al.* (2), since it is believed that generic differentiation should not be based solely upon this one biochemical activity.

The organisms are described below in respect of morphology and characters on nutrient media, a summary of all characters that would admit of tabular treatment are shown in Table I. Characters common to all of the strains are as follows: all are able to grow anaerobically and are motile, Gram-negative, and do not produce indol or hydrogen sulphide; only *V. perimastix* and

B. effluens reduce nitrate to nitrite; and traces of ammonia were found in old cellulose cultures of *B. vagans* and *B. Kellermani;* none produced ammonia from urea.

From the table of biochemical characteristics it might be held that the species numbered 4 and 5 should be considered as varieties of one species, the sole difference between them being that the strains of *B. Kellermani* (No. 5) did not decompose dulcitol; reference to their cultural characters on agar would, however, suggest that they differ sufficiently; they are therefore tentatively proposed as separate species.

Photographic reproductions of camera lucida drawings of cell morphology, flagella, and spores, and of growth upon agar plates, appeared as plates in the senior author's thesis (Alarie, 1).

Species 1—Vibrio perimastix n. sp.

(The specific name is derived from the Greek, *peri*, around, and *mastix*, whip)

Cell form: curved rods, $0.4-0.7\mu$ by $1.4-5.6\mu$ with 8 to 20 peritrichous flagella.

Agar colony: spreading, irregular, flat, bluish (barely visible), butyrous; 8 to 12 mm. in 24 hr.; similar on starch and dextrose agars.

Agar slant: thin, colorless to grayish, curly, barely visible, butyrous.

Nutrient broth: no visible growth.

Gelatin stab: no liquefaction.

Potato: good filiform growth, light yellow, shining.

Litmus milk: no change.

Cellulose decomposition: with peptone, paper broken in three or five days; with nitrate in eight or nine days; on cellulose agar the growth is spreading and the hydrolysis is superficial.

Source: isolated from a muck soil.

Species 2—Vibrio hyperion n.sp.

(Specific name from the Greek, Hyperion, the sun-god; in allusion to the flame-shaped projections of the colonies)

Cell form: short curved rods, $0.4-0.7\mu$ by $1.4-2.4\mu$; a few cells 4.2μ long; with one polar flagellum.

Agar colony: on starch agar, spreading, with curly projections, irregularly raised, shining, bluish gray, butyrous; curling less on dextrose agar, and colonies yellowish gray.

Agar slant: filiform, with some curling; shining, yellowish gray, semiopaque; old cultures yellow.

Nutrient broth: no visible growth.

Gelatin stab: no liquefaction.

Potato: good filiform growth, buff, shining.

Litmus milk: no change.

Cellulose decomposition: with peptone, paper broken in five or seven days, and tinged with yellow; liquid yellow in old cultures; with nitrate, paper broken after three weeks or longer. Well defined hydrolysis on cellulose agar.

Source: from an unfertilized soil under pasture.

Species 3—Bacterium infirme n.sp.

(Specific name from the Latin, infirmus, weak)

This organism was not viable after some months.

Cell form: straight rods; a few curved; $0.5-0.7\mu$ by $1.7-2.8\mu$, some up to 6.3μ; single or paired; with 5 to 15 peritrichous flagella.

Agar colony: 3 to 4 mm., white, circular, umbonate to convex, smooth, translucent, butyrous.

Agar slant: filiform, grayish, translucent, butyrous; occasionally with gas in the butt.

Nutrient broth: turbid, with white slimy sediment.

Gelatin stab: no liquefaction; gas with starch–peptone.

Potato: filiform, scanty, buff, shining, dull white border.

Litmus milk: acid.

Cellulose: with peptone, paper broken in 17 to 19 days; with nitrate, in 30 days; on agar, decomposition not visible.

Source: muck soil, with *V. perimastix.*

Species 4—Bacillus vagans n.sp.

(The specific name is Latin, wandering, in allusion to the spreading growth on cellulose agar)

Cell form: straight rods, $0.7-0.8\mu$ by $1.4-4.2\mu$, single or paired; with 5 to 15 peritrichous flagella; spores central to subcentral; slightly broader than the cell.

Agar colony: 2 to 3 mm. circular, slightly convex, bluish, shining, translucent, butyrous.

Agar slant: filiform, grayish, shining, translucent, gummy.

Nutrient broth: slightly turbid; a scanty viscid sediment.

Gelatin stab: funnel-shaped liquefaction.

Potato: filiform, light yellow, shining.

Litmus milk: slightly acid.

Cellulose: paper broken in 8 to 10 days; spreading colonies on agar decomposed cellulose without clear enzymic zones.

Source: two strains from the organic matter layer of a podsol soil under forest cover.

Species 5—Bacillus Kellermani n.sp.

(Specific name, in honour of Dr. K. F. Kellerman)

Cell form: straight rods, with squared ends, 0.7—0.8μ by 1.0—3.5μ, single or paired; flagella 5 to 15, peritrichous; spores central to subcentral, slightly broader than the cell.

Agar colony: 2.5 to 3.5 mm., nearly circular, white, umbilicate, shining, gummy.

Agar slant: filiform, cream-colored or white, shining, opaque, gummy.

Nutrient broth: slightly turbid, white sediment.

Gelatin stab: stratiform liquefaction.

Potato: filiform, white, dull.

Litmus milk: slightly acid.

Cellulose: with peptone, paper broken in seven days; with nitrate, in 16 or 21 days; decomposition on agar not visible.

Source: two strains were isolated, from two different pasture soils that had not been fertilized.

Species 6—Bacillus soli n.sp.

(The specific name is Latin, *solum*, the soil)

Cell form: straight, a few curved rods; 0.7—0.8μ by 1.4—3.5μ, single cells, with 4 to 12 peritrichous flagella; spores terminal.

Agar colony: 2 to 5 mm., nearly circular, grayish to white, convex, shining, translucent, butyrous.

Agar slant: filiform, grayish, shining, translucent; gas in the butt.

Nutrient broth: turbid, white slimy sediment.

Gelatin stab: no liquefaction; with starch the gel is broken by gas.

Potato: filiform, buff, shining, with dull white border.

Litmus milk: litmus reduced.

Cellulose: with peptone, paper broken in 21 days; with nitrate in 30 days; no enzymic clear zone on agar.

Source: from a field under oats that had not been fertilized.

Species 7—Bacillus torquens n.sp.

(Specific name from Latin, *torquere*, to twist, in allusion to the manner of growth on agar plates)

Cell form: short straight and curved rods, 0.5—0.7μ by 1.4—2.1μ, with 4 to 16 peritrichous flagella; spores terminal or subterminal, centrally placed and arising from a 'sporocyst' in clostridium cells formed on starch; these latter stain deeply at one end, and measure 0.8—1.4μ by 2.1—4.9μ.

Agar colony: motile by threadlike projections up to 25 mm. long, where new colonies form; grayish, shining, butyrous.

Agar slant: filiform, with some projections, yellowish gray, shining, semi-opaque, butyrous, later slimy and agar tinged with yellow.

Nutrient broth: no visible growth.

Gelatin stab: no liquefaction.

Potato: filiform, buff, shining.

Litmus milk: no change.

Cellulose: with peptone, paper broken in four to seven days; with nitrate, no decomposition; the paper and, later, the fluid tinged with yellow; a well defined enzymic clear zone on agar, the fibres being completely decomposed.

Sources: three strains from a field of potatoes that had been fertilized.

Species 8—Bacillus effluens n.sp.

(Specific name from Latin, *effluere*, to flow outwards, in allusion to the swarming projections from the colonies)

Cell form: curved rods, $0.5-0.8\mu$ by $1.4-4.2\mu$, single; on starch agar developing to clostridia $0.9-1.4\mu$ by $2.8-5.6\mu$ staining deeply at one end; with 5 to 16 peritrichous flagella; spores terminal or subterminal in both forms of cell; no sporocysts.

Agar colony: with *starch*, 1 to 4 mm. circular, convex, white, shining, gummy, occasionally swarming; with *dextrose*, yellowish white, extensive swarming by one strain, less with the other, projections up to 25 mm.

Agar slant: beaded to filiform, margin sometimes with curling projections, gray, tinged with yellow; when older, shining, opaque, gummy, firmly adherent.

Nutrient broth: no visible growth.

Gelatin stab: no liquefaction.

Potato: scanty growth, light yellow, shining.

Litmus milk: no change.

Cellulose: with peptone, paper broken in 7 to 10 days; with nitrate in 14 days; growth spreading on agar, without a clear zone.

Source: two strains from a field under potatoes that had been fertilized.

Conclusions

From the results of this study, it is evident that starch is well suited for the isolation of cellulose decomposing bacteria. If not all cultures were pure at the first isolation, it was relatively easy to purify them by means of dilution followed by a second plating. Difficulties were met with only in the purification of *B. torquens* as it was thought that the slimy cultures would carry in the dilution some cells of *V. hyperion* to which it is morphologically very similar in young cultures, and in physiological characteristics when older. Plating on starch agar of dilutions in distilled water after treatment for spores

TABLE I

ACTION OF THE BACTERIA ON CARBOHYDRATES, ALCOHOLS, AND GLUCOSIDES. O = NO CHANGE; ± = WEAKLY ACID; + = ACID; ++ = ACID WITH GAS

Species	Arabinose	Rhamnose	Xylose	Glucose	Fructose	Galactose	Mannose	Lactose	Sucrose	Maltose	Trehalose	Cellobiose	Raffinose	Melezitose	Starch	Inulin	Dextrin	Glycogen	Glycerol	Mannitol	Sorbitol	Inositol	Dulcitol	Salicin	Esculin
1. *V. perimasitx*	±	O	±	±	±	±	±	±	±	±	±	±	±	±	±	O	±	±	O	O	O	O	O	±	±
2. *V. hyperion*	±	O	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	O	O	O	O	±	±
3. *B. infirme*	+	±	+	+	+	+	+	+	+	+	+	±	+	+	+	±	+	+	±	O	O	±	±	O	+	+
4. *B. vagans*	+	O	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	O	O	±	±	+	+
5. *B. Kellermani*	+	O	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	O	O	±	O	+	+
6. *B. soli*	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	±	±	±	±	+	+
7. *B. torquens*	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	O	O	O	O	±	±	±
8. *B. effluens*	±	O	±	±	±	±	±	±	±	±	±	±	±	±	±	O	±	±	O	O	O	O	O	±	±	±

(10 to 12 min. heating at 80° C.) finally gave a pure culture. No other difficulties were met with in the isolations.

As was expected, no specialized cellulose decomposing organisms were found, but the organisms isolated exhibit a great variety in their activities, some breaking the filter paper strip in mineral salt solution in three or seven days, as was the case with *V. perimastix*, and *B. torquens*, while some others, e.g., *Bacterium infirme* and *Bacillus soli*, developed that far only after three weeks. The fact also that the organisms isolated on starch and described as cellulose decomposers grow on many carbohydrates indicates that the versatile cellulose-hydrolyzing microflora is well distributed.

It may be said against the use of starch in the isolation of cellulose decomposing bacteria that the scheme of isolation is long and furthermore that keeping the stock cultures on starch will cause the organisms to loose their ability to decompose cellulose. To the first objection it may be stated that the procedure is not longer than any other since pure cultures are very readily obtained. But what is likely to happen, in the elective cultures, is that the active cellulose decomposing bacteria, having a weaker diastatic power, may be ruled out in numbers by the strongly diastatic bacteria of weaker activity in the cellulose breakdown. This may well be the limiting condition of this method. As to the loss of activity of the bacteria when incessantly grown on starch, Simola (24) attributed this phenomenon to the fact that mineral and not organic nitrogen was fed to the bacteria. The organisms described in the present work have been grown on starch peptone mineral salt agar slants and their ability to retain their cellulolytic ability was confirmed in studies made some months later.

It may also be advocated in favor of the use of starch that its composition is more uniform than that of the fractions obtained by the hydrolysis of cellulose by strong acids.

The *Bacillus* group is reported officially here for the first time for Quebec soils, though some previous unpublished investigations by the junior author had shown that species of this genus capable of decomposing cellulose were present; the present work, indeed, has been based upon those findings.

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AN INDICATOR AGAR FOR THE DETERMINATION OF THE RELATIVE CONCENTRATION OF ASCORBIC ACID IN POTATO TUBER TISSUE[1]

By Wm. Newton[2]

Abstract

An indicator agar that reveals the approximate concentration of ascorbic acid in potato tuber tissue by direct contact is prepared by heating together 2 gm. agar, 75 ml. water, 10 ml. of a 10% solution of potassium iodide, and 5 ml. of a 1% solution of soluble starch. After cooling to 60° C., 5 ml. of glacial acetic acid and 5 ml. of an 0.02 *N* solution of potassium iodate are added and the mix is immediately poured into Petri dishes. When uniform filter paper discs saturated with standard ascorbic acid solutions or uniform tuber tissue discs are placed upon the agar surface, after 24 hr. at 5° C., the size of the area decolorized is directly related to the concentration of ascorbic acid in the solutions and tissue discs. Tissue discs from tubers affected with mosaic and leaf roll decolorize a greater area than discs from normal tubers from the same variety, and the decolorization is independent of tuber size. The diagnostic accuracy of the method is further strengthened by the tendency of virus infected tissue to remain white and healthy tissue to become blue-black.

Introduction

Indicator agars were studied in the hope of developing a simple means of utilizing the ascorbic contents of plant tissue as indices of virus disease infection. Smith and Paterson (2) have shown that potato tubers infected with mosaic and leaf roll contain higher contents of ascorbic acid than tubers from healthy plants of the same variety, as judged by the titration values of trichloroacetic acid extracts with the dye 2,6-dichlorophenolindophenol. These authors have shown also that within the same variety the variation in ascorbic acid between tubers is relatively small and appears to be independent of the size of the tubers and the manuring of the crop. On the other hand, they have shown that there is a loss of ascorbic acid during storage, and the loss probably varies with the storage environment. A preliminary study of three potato varieties grown in British Columbia, viz., Netted Gem, Burbank, and White Rose, has confirmed the conclusion of these authors that within a single variety an abnormally high content of ascorbic acid in the tuber tissue is a reliable index of virus infection. Simplification of the means of determining relative concentrations appeared possible when it was discovered that, if uniform tuber tissue discs are placed upon agar surfaces, the ascorbic acid diffuses into the agar in amounts roughly proportional to the concentration within the tissue.

The Indicator Agar Method

The indicator agar is prepared by heating together 2 gm. agar, 75 ml. water, 10 ml. of a 10% solution of potassium iodide, and 5 ml. of a 1% solution of

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soluble starch. When the agar dissolves and the mix is cooled to approximately 60° C., 5 ml. of glacial acetic acid and 5 ml. of an 0.02 *N* solution of potassium iodate are added, and the indicator agar is immediately poured into Petri dishes, 10 ml. per 9 cm. dish, or proportionately greater amounts when larger dishes are used. Care must be taken that the depth of the agar is uniform. Some Petri dishes are unsuitable because the bottoms are not flat. The cooling of the agar solution prior to the addition of potassium iodate is essential. If the iodate is added at a temperature close to the boiling point, the iodine that is formed by the reaction of the constituents is lost, and the characteristic starch–iodine blue color of the indicator agar will not form. The Petri dishes are stored in a refrigerator until used, as it is assumed that storage at low temperatures preserves the sensitivity of the agar.

The sensitivity of the agar is measured by placing filter paper discs saturated with ascorbic acid solutions on the agar surface. The diameter of the decolorized zone, the halo around the discs, is a function of ascorbic acid concentration, the depth of the agar layer, and the concentration of the potassium iodate; consequently, if the depth of the agar is constant and the loss of iodine is negligible in the preparation of the indicator agar, the diameters of the halos are the same after 24 hr. at 5° C. when the filter paper discs are saturated with a standard ascorbic acid solution and are placed upon the indicator agar surfaces.

Tissue cylinders are removed from the potato tubers by means of a cork borer and discs approximately 5 mm. in thickness are cut therefrom by means of a razor blade. These are placed immediately on the surface of the indicator agar and the dishes are returned to the refrigerator. Discs from healthy tubers are placed in the same dishes with those from the corresponding variety infected with mosaic or leaf roll. The diameters of the halos are noted after 24 hr. at 5° C. The tests are carried out at 5° C. chiefly because of convenience. At this temperature the decolorization of the agar is slowed down sufficiently to avoid error through the practical difficulty of timing correctly the period that the discs are in contact with the agar. However, the conduct of the tests at higher temperatures has the advantage of speed. At 25° C. within two hours the halos surrounding discs from tubers affected with mosaic or leaf roll are appreciably larger than those around the discs from healthy tubers of the same variety.

Results

When uniform tuber tissue discs of the potato varieties Netted Gem, Burbank, and White Rose were placed upon the indicator agar, after 24 hr. the halos were invariably larger around the discs derived from plants infected with leaf roll or mosaic than from healthy plants of the same variety. Typical examples are illustrated in Fig. 1, A to D.

In addition to the contrast in halo size, the upper surfaces of the discs derived from virus infected plants tended to remain wholly or partially white, while those from the tubers of healthy plants became blue-black. The

contrast in color and halo size is shown in Fig. 1, A. The blue-black color of the discs from healthy plants is probably caused by the volatile iodine.

Tissue from tubers infected with leaf roll apparently contains less ascorbic acid when derived from old than from new tubers of the same variety. The contrast is illustrated in Fig. 1, E, and represents the effect of discs from the 1946 and 1947 tuber crops.

The halo diameters as induced by discs from healthy tubers were approximately the same regardless of tuber size within a single variety (Fig. 1, F).

The indicator agar served well as a rapid method of determining the approximate concentration of ascorbic acid in miscellaneous fluids. The concentration in fluids was determined by comparing the size of the decolorized zones induced by single drops of standard ascorbic acid solutions and the unknowns, or by uniform filter paper discs saturated with the standards (Fig. 1, G) and the unknowns. The effect of adding a single drop of a standard ascorbic acid solution containing 50 mgm. per 100 ml. compared with a single drop of lemon juice is shown in Fig. 1, H. The lemon juice contained approximately 50 mgm. per 100 ml. as judged by the similar diameters of the decolorized areas.

Permanent records are conveniently obtained in the form of negative prints by placing the dishes in a darkroom on photographic paper, followed by exposure to light. The illustrations in Fig. 1 were obtained in this manner, with the exception of A and H, which were photographed in the ordinary manner to illustrate the typical blue-black color acquired by potato discs from healthy tubers in contrast with virus infected, and the influence of lemon juice in contrast with a standard ascorbic acid solution.

Discussion

The preparation of the indicator agar was based upon an iodometric method for the chemical determination of ascorbic acid in plant tissue (1). Iodometric methods are not so popular as those based upon the reduction by ascorbic acid of the dye 2,6-dichlorophenolindophenol. Agars were prepared that contained this dye at a wide range of concentrations and as adjusted to a wide range of hydrogen ion concentrations, but none revealed the relative concentrations of ascorbic acid in potato tuber tissue discs so satisfactorily as the iodometric indicator agar described in this paper. At low concentrations the fading of the dye made it difficult to distinguish the decolorized area from the non-reduced agar zone. At higher concentrations the dye diffused back into the decolorized zone after it had reached its maximum size through the exhaustion of the ascorbic acid by oxidation. With the iodometric indicator agar, the decolorized zones remained constant after they had reached their maximum size through the exhaustion of the ascorbic acid.

The indicator agar is particularly useful in the diagnosis of leaf roll. Under conditions of drought, potato inspectors frequently have difficulty in distinguishing healthy plants from those infected with leaf roll, owing to the tendency of the leaves of healthy plants to curl when they lack moisture.

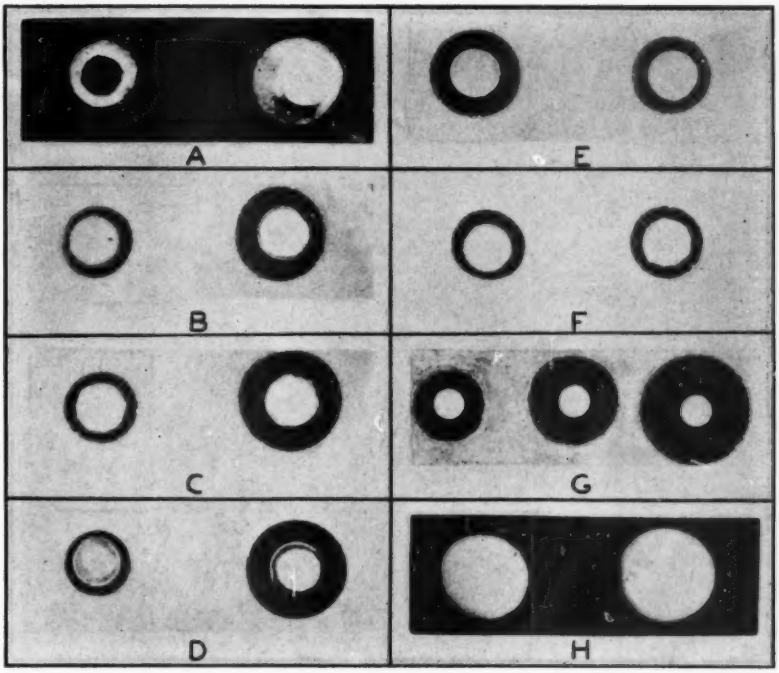

FIG. 1. *The decolorization of indicator agar by tissue discs cut from potato tubers and by ascorbic acid solutions. A. Tuber discs from variety Burbank—left, healthy; right, affected by leaf roll. B. From variety Burbank—left, healthy; right, affected by mosaic. C. From variety White Rose—left, healthy; right, affected by leaf roll. D. From variety White Rose—left, healthy; right, affected by mosaic. E. From variety Netted Gem affected by leaf roll—left, 1947 tuber; right, 1946 tuber. F. From variety Netted Gem—left, healthy 2 cm. tuber; right, healthy 10 cm. tuber. G. Filter paper discs saturated with standard ascorbic acid solutions—left, 25 mgm. per 100 ml.; centre, 50 mgm. per 100 ml.; right, 100 mgm. per 100 ml. H. Left, one drop lemon juice; right, one drop standard ascorbic acid solution, 50 mgm. per 100 ml.*

Although no difficulty has been experienced in distinguishing leaf roll or mosaic in tubers derived from infected planting stock, no evidence has been obtained that virus infection can be detected by the ascorbic acid content of the tubers when the infection has occurred late in the growing season.

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STUDIES ON THE DECOMPOSITION OF CELLULOSE BY MICRO-ORGANISMS[1]

By A. S. Perlin,[2] M. Michaelis,[3] and W. D. McFarlane[4]

Abstract

The metabolism of an impure culture of an aerobic cellulose decomposing bacterium, *Vibrio perimastix*, was studied. The products of cellulose decomposition included carbon dioxide, a pigment resembling riboflavin, a bacterial polysaccharide, and traces of acid. Carbon dioxide was found to be essential for the decomposition of cellulose and could not be replaced by calcium carbonate. Increasing the carbon dioxide content of the air above 1.2% retarded growth on a glucose medium.

Respiration studies were carried out using cellulose, glucose, and cellobiose as substrates, and the possible role of the latter two as intermediates in cellulose decomposition was investigated. Glucose was produced from cellulose when toluene was added to cultures during active decomposition; evidence is presented that a dialyzable factor produced by the bacteria is essential for glucose formation. Phosphorylation inhibitors prevented growth of the bacteria on cellulose, glucose, and cellobiose, inhibited respiration of active cellulose cultures, and retarded the production of glucose by toluene-treated cultures. Cellulose treated with alkali to increase the proportion of amorphous to crystalline cellulose was more rapidly decomposed than untreated cellulose.

Introduction

Cellulose is decomposed to simple chemical compounds by micro-organisms in a variety of natural processes. In this manner, a large part of the carbon of decaying vegetation is reconverted into forms utilizable by other living organisms, and a proper balance of the carbon cycle is maintained. However, very little is known about the micro-organisms involved or the mechanism by which the decomposition is accomplished.

This paper reports a study of the decomposition of cellulose by *Vibrio perimastix* (1), associated with an unidentified bacterium. The primary objective of this investigation was to obtain information regarding the enzymes active in the decomposition of cellulose by aerobic, mesophilic bacteria.

Materials and Methods

I. Media

Salt Solution

The salt solution used was similar to that of Kalnins (5), and consisted of: 0.5 gm. disodium phosphate; 0.5 gm. monopotassium phosphate; 0.2 gm. magnesium sulphate septahydrate; 0.1 gm. calcium chloride dihydrate; 0.1 gm. sodium chloride; 0.02 gm. ferric chloride, and 1.0 gm. sodium nitrate

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per litre of distilled water. The hydrogen ion concentration was approximately pH 6.6. When other hydrogen ion concentrations were desired the amounts of the individual phosphate salts were varied accordingly, but the total phosphate was always 0.1%.

Cellulose

Whatman No. 41 filter paper was used as the cellulose substrate.

Cellulose Broth

This consisted of 1 gm. of cellulose per 100 ml. salt solution.

Cellulose Agar

Prepared according to Kalnins (5).

Starch, Glucose, and Cellobiose Broths

These consisted of 1 gm. of the appropriate carbohydrate per 100 ml. salt solution. In the case of the latter two, the sugars were autoclaved in distilled water and sterilized salts then added.

Starch–Peptone Agar

Prepared by adding 1.5% washed agar and 1% peptone to starch broth.

II. The Culture

The culture appeared to contain only one organism, also a *Vibrio*, in association with the *V. perimastix*. The impure culture was used in preference to a pure culture of *V. perimastix* since it was considerably more active in decomposing cellulose, and at the same time gave reproducible results. The bacteria were isolated from the soil (1).

Cellulose was actively decomposed in cellulose broth over a temperature range of 20° C. to 40° C. and at hydrogen ion concentrations from pH 5.0 to pH 7.5. The decomposition was accompanied by the production of a greenish-yellow pigment, the reduction of nitrate to nitrite, and a marked rise in the pH of the culture medium.

On starch–peptone agar the organisms produced a heavy, slimy growth, and the starch was partially hydrolyzed. This medium was used to maintain stock cultures, transfers being made periodically from cellulose medium. Inoculations from such cultures were as effective in attacking cellulose as those from cellulose broth. Heavy growth was also obtained with starch and glucose broths. There was only limited activity on cellulose agar, the clear zonations surrounding the colonies being very narrow.

III. Procedure

Cellulose broth cultures of the organisms, contained in 1500 to 2000 ml. Erlenmeyer flasks, were aerated on a rotary shaker at 26.6° C. The initial hydrogen ion concentration was varied from pH 6.2 to 6.7.

After fermentation for 10 to 15 days the culture was filtered through a Whatman No. 42 filter paper. The residue was washed with hot water, alcohol, and ether, and dried to constant weight at 80° C.

The filtrate was tested for reducing sugars with Fehling's solution, and for pentoses or uronides by the phloroglucinol reaction. Ether extraction was used for the detection of acids, and steam distillation for alcohol. To precipitate mucilaginous materials, a portion of the filtrate was treated with acidified alcohol, according to Walker and Warren (14), or with dilute hydrochloric acid, according to Fåhraeus (4).

Experimental Results

I. Products of Cellulose Decomposition

Reducing sugars were not found in any of a large number of cultures examined. A positive phloroglucinol reaction was always given; the original filter paper gave a negative test. Acid distillation into phloroglucinol and hydrochloric acid gave a weak test for furfural, suggesting the possible presence of uronic acids. Traces of acid were detected but the amount was too small to permit identification even when a litre of culture fluid was taken for the test.

Small amounts of a white, bulky precipitate, which resembled the "mucilaginous material" of Walker and Warren (14), were separated from the filtrate by alcohol and by acid precipitation. However, the material gave a negative test for uronic acids and hence was not identical with the precipitate that they obtained. The material was insoluble in hot water, dilute acid, and alkali but dissolved slowly in 60% sulphuric acid and was reprecipitated by diluting the solution with water, thus resembling cellulose.

Accordingly, a comparison was made of the rates of acid hydrolysis of the precipitated material and of very fine shreds of cellulose that had also been recovered from the fermentation. The precipitate was dried, and ground to a fine powder. It was then suspended in acetone to lyse adhering bacterial cells, and the acetone was removed. This was followed by extraction in the cold (3° C.) with 5% trichloroacetic acid and another washing with acetone, and the material was then dried *in vacuo*. The residual cellulose received a similar treatment with trichloroacetic acid.

The samples were hydrolyzed on a steam-bath with 2*N* hydrochloric acid, and the reducing sugar content of aliquot portions removed at intervals was determined according to Shaffer-Somogyi (12). The results given in Table I indicate more rapid hydrolysis with the precipitated material.

TABLE I

RATES OF HYDROLYSIS OF ACID PRECIPITATED AND RESIDUAL CELLULOSE

Time, min.	Precipitate (Mgm. glucose per ml.)	Cellulose (Mgm. glucose per ml.)
15	0.44	Trace
30	0.54	0.28
60	0.56	—
120	0.63	0.45
180	0.73	0.45

Note: The purified material contained 7.2% nitrogen.

Pigment

A greenish-yellow, fluorescing pigment resembling riboflavin was present in all cultures. This pigment could not be extracted with ethyl ether, petrol ether, chloroform, or ethyl acetate, but was extracted by benzyl alcohol in the presence of barium chloride (3), and by butyl alcohol saturated with sodium sulphate (8). It was adsorbed on freshly prepared lead sulphide and eluted with a mixture of 70 parts water, 2 parts glacial acetic acid, and 30 parts pyridine (8)—solvents used for extraction of riboflavin. These extracts showed the characteristic fluorescence of riboflavin, the presence of which was also demonstrated by a microbiological assay on the culture liquor (11).

The intensity of the fluorescence was diminished rapidly by ultraviolet radiation, by reduction with sodium hyposulphite, and to a lesser degree by oxidation with permanganate. The light absorption curve of the pigment in butyl alcohol showed a sharp rise in the range 4300 to 4000Å.

Carbon Dioxide

An apparatus was designed for the gravimetric determination of the carbon dioxide produced in the cellulose decomposition. The carbon dioxide in the air entering the culture was removed by passage through a 50% solution of sodium hydroxide and then through soda lime and the carbon dioxide evolved in fermentation was absorbed on ascarite. All newly inoculated cultures failed to grow in this apparatus and it was believed that this could only be due to the removal of carbon dioxide from the incoming air. It was, therefore, decided to investigate the carbon dioxide requirements of the organisms.

II. Role of Carbon Dioxide

An experiment showing that carbon dioxide is essential for the decomposition of cellulose by the organisms has already been described (10). Four culture flasks containing cellulose broth received a very small inoculum; two were aerated with normal air and two with carbon dioxide-free air. Inhibition of growth in the absence of carbon dioxide was also produced in the same manner with glucose and cellobiose as substrates. The addition of 1% calcium carbonate to glucose permitted growth in the flasks aerated with carbon dioxide-free air, but it was unsatisfactory for growth on cellulose. It has, therefore, not been possible to procure the necessary data for a carbon-balance sheet of cellulose decomposition.

The effect of concentrations of carbon dioxide above that of normal air was investigated using glucose broth as the substrate. An aspirator of the common type, with a capacity of 48 litres, was employed, and dilute acid was used as the displacing fluid. A blank determination was always included, the rates of bubbling being kept relatively constant for all tubes by means of precision pinchcocks. Growth was measured nephelometrically with an Evelyn colorimeter employing the 520 filter. A small inoculum was always used, the age of which was approximately the same for each trial.

The concentrations of carbon dioxide were varied between 1.2 and 10%. It was found that concentrations of 2.5 to 10% retarded growth but at 1.2% carbon dioxide, the growth was equal to that in air.

III. The Influence of the Physical Nature of the Cellulose

The modern concept of the structure of cellulose holds that the closely-packed nature of the crystalline portion of cellulose renders difficult the penetration of chemical and physical agents between the micelles. On the other hand, the amorphous portion is more readily affected by these same agents. The latter should, therefore, be more accessible to bacterial enzymes.

Accordingly, cellulose was treated to increase its content of the amorphous portion, and the rate of decomposition compared with untreated cellulose. The treatment was carried out according to Assaf *et al.* (2), with the exception that 13% sodium hydroxide was used, since it was found to be the minimum concentration of alkali that would satisfactorily swell the cellulose.

A set of 200 ml. Erlenmeyer flasks containing the alkali-treated cellulose in salt solution was inoculated and cultured together with a set containing untreated cellulose. The results are illustrated in Fig. 1. There was more rapid decomposition of the alkali-treated cellulose, and these cultures produced considerably more pigment than the cultures containing untreated cellulose.

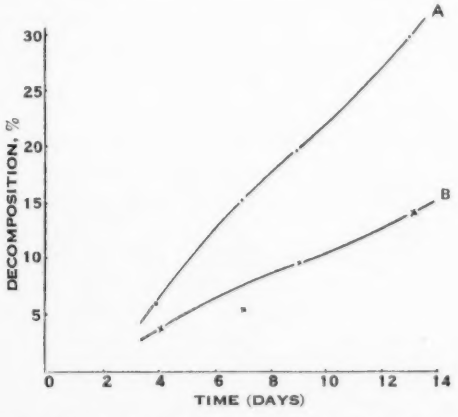

FIG. 1. *Influence of the physical nature of the substrate on the decomposition of cellulose.*
A – Treated cellulose.
B – Untreated cellulose.

IV. Observations on Respiratory Enzymes

The Thunberg and Warburg techniques were employed at a temperature of 27° C. The first experiments were carried out with resting bacteria grown on starch or glucose broth. The Thunberg technique showed dehydrogenase activity with glucose and cellobiose, the former requiring slightly less time to decolorize the methylene blue. Cellulose prolonged the decoloration time.

Similar results were obtained by the 'direct' method of Warburg: the oxygen uptake with glucose as the substrate was slightly higher than with cellobiose, and cellulose gave negative results.

Cultures that were actively decomposing cellulose showed an oxygen uptake that was readily measurable with the respirometer. In this case samples of the cellulose with adhering bacteria were removed from the cultures with wide-mouth pipettes and used directly in the Warburg vessels, the hydrogen ion concentration of the cultures being first readjusted to about pH 6.5.

Effect of Carbon Dioxide

The respiration of a 17 day old cellulose culture was measured by both the 'direct' and 'indirect' Warburg methods, that is, in the presence and in the absence of carbon dioxide. The results, illustrated in Fig. 2, show that the total oxygen uptake was greater in the presence of carbon dioxide.

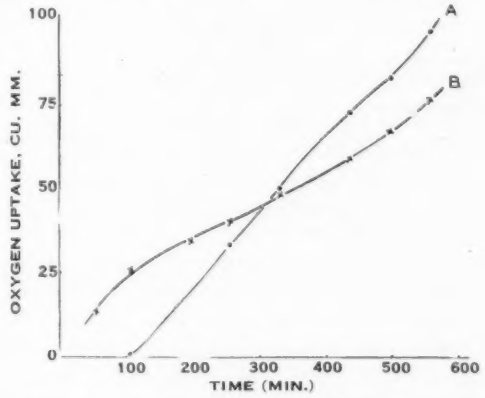

FIG. 2. *Influence of the presence of carbon dioxide on the oxygen uptake of cellulose cultures.*

A – Carbon dioxide present.
B – Carbon dioxide absent.

Effect of Adding Postulated Intermediates

The effect of adding the postulated intermediates of cellulose decomposition—glucose and cellobiose—to cellulose was next investigated by the indirect method. The blank oxygen uptake of the cultures was determined for each vessel over a period of two to three hours so as to allow for differences in uniformity of the suspensions in different vessels. The substrates to be tested were then added from the side-arms, their final concentrations being about 1%. The results of one such experiment are given in Fig. 3. The bacteria–cellulose suspensions used in this experiment were from a 20 day culture. The stimulatory effect of glucose and the more marked stimulation by cellobiose were also observed by the direct method, and at 27° C. as well as at 37° C. Similar experiments with younger cultures, about eight days, did not show appreciable differences between the substrates added to cellulose.

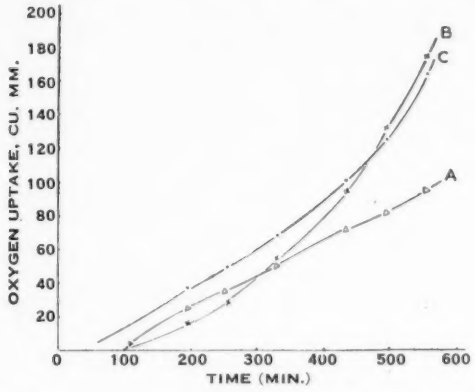

FIG. 3. *Effect of the presence of glucose or cellobiose on the oxygen uptake of cellulose cultures.*

A – Cellulose only.
B – Cellobiose added.
C – Glucose added.

V. Production of Reducing Sugars

A number of workers have demonstrated the formation of reducing sugars in cellulose cultures by employing various treatments designed to disrupt the normal activity of the organism (9). In some cases, however, these methods have failed to cause an accumulation of reducing sugars. The ability of the bacteria, used in this study, to produce reducing substances from cellulose was therefore examined.

Aliquots of cellulose cultures showing normal activity were adjusted to about pH 6.5, 5 ml. of toluene per 50 ml. of culture fluid were added, and the cultures were incubated at 26.6° C. Aliquots were removed periodically and reducing sugars determined by the Shaffer–Somogyi method.

The amount of reducing sugars varied considerably with the age and final hydrogen ion concentration of the cultures. In some cases the rate of formation was rapid and the final concentration of sugar was of a high order. For example, one culture showed an accumulation of 56.3 mgm. of reducing sugars per 50 ml. after four days' incubation.

(*a*). *Identification of the Reducing Sugar*

About 500 ml. of an active cellulose culture was incubated with toluene for five days, the cellulose filtered off and the culture liquid concentrated *in vacuo* to a small volume. To precipitate inorganic salt, 95% ethyl alcohol was added, the alcohol was distilled off, and the remaining liquid was clarified with charcoal. This solution yielded an osazone when heated for about 45 min. with phenylhydrazine hydrochloride and sodium acetate, the crystals having the characteristic appearance of glucosazone; only one type of crystal was present. Melting-point determinations were carried out with the unknown

osazone and glucosazone prepared from free glucose by the same method. Both gave melting points of 203° to 205° C., corrected (theoretical 204° to 205° C.). A mixed melting-point carried out with equal portions of the unknown and glucosazone gave a value of 205° C., corrected. The reducing sugar produced in the cellulose cultures was, therefore, glucose.

(*b*). *Effect of Dialysis on the Formation of Glucose*

The rate of glucose formation was generally found to decline gradually after a few days, and in some cases, dropped off sharply. The possibility was considered that accumulation of glucose might retard the additional formation of glucose by a mass-action effect. Dialyzing the sugar as rapidly as it is formed should, therefore, increase the total sugar produced over a given period.

Accordingly, aliquots of active cellulose cultures that were treated in the usual manner with toluene, were dialyzed in collodion sacs against a salt solution of equal salt concentration and the same hydrogen ion concentration; toluene was also added to the dialyzate.

Dialyzing 50 ml. aliquots of cellulose culture against 100 ml. and 200 ml. portions of salt solution did not show a significant increase in the total glucose produced in 4.5 days. The values obtained were 56.4 mgm. and 56.3 mgm., respectively, as compared with 53.6 mgm. produced by 50 ml. of the same culture incubated with toluene in an Erlenmeyer flask.

When dialysis was carried out in like manner against a larger volume of salt solution (400 ml. or 500 ml.) no reducing sugars were produced in the same period of time. In a further experiment, 50 ml. aliquots of a culture treated with toluene were dialyzed against 100 ml., 300 ml., and 500 ml. portions of salt solution, respectively. For comparison, another aliquot was incubated in an Erlenmeyer flask. The rates of glucose formation were determined with the results given in Table II.

TABLE II

EFFECT OF DIALYSIS ON THE FORMATION OF GLUCOSE

(Total mgm. glucose, including dialyzate)

Time, days	Untreated	Dialyzed against		
		100 ml.	300 ml.	500 ml.
1	11	0	0	0
2	20	21	Trace	0
4	29	27	33	0
5	—	43	40	—

These results suggested that some substance(s) produced by the bacteria, and necessary for glucose production was being removed by dialysis; and that, for glucose formation a sufficient concentration of the dialyzable factor

must be present to counteract the effect of dialysis. The hydrogen ion concentration inside the collodion membranes did not change appreciably and hence a Donnan equilibrium effect was considered unlikely.

Should a dialyzable factor be involved, then on discontinuing the dialysis the concentration should increase and glucose formation be resumed. To investigate this possibility, a culture that had been dialyzed against 500 ml. of salt solution and in which no glucose had been formed was divided into two portions. These were incubated with toluene and after 12 hr. reducing sugars were found to be present, the concentration being 0.2 mgm. per ml.

Further evidence that a dialyzable factor is involved in glucose formation was obtained as follows:

Two 50 ml. aliquots of a toluene-treated culture were dialyzed against 100 ml. and 300 ml. of salt solution, respectively. When the presence of glucose was detected in the 100 ml. dialyzate, equal portions of the culture that was being dialyzed against 300 ml. of salt solution and that contained no glucose were transferred to two test tubes. To Tube I was added 10 ml. of the 100 ml. dialyzate, which should have contained an appreciable concentration of the dialyzable factor, and to Tube II was added 10 ml. of a freshly-prepared salt solution. After eight hours' incubation, Tube I contained 3.5 mgm. glucose, whereas glucose was not present in Tube II. At 22 hr. the concentration in Tube I had increased to 4.6 mgm. and only a trace appeared in Tube II.

VI. Optimum Hydrogen Ion Concentration for Activity with Cellulose, Glucose, and Cellobiose as Substrates

If glucose and cellobiose are intermediates in the decomposition of cellulose, then it would be expected that the optimum pH would be the same for cellulose decomposition and for growth on glucose and cellobiose. Accordingly, the optimum pH for activity on these three substrates was determined.

The hydrogen ion concentration of the glucose and cellobiose broths was adjusted with $M/15$ phosphate buffer; the usual amounts of the other salts were used. On cellulose, growth was not obtained with $M/15$ buffer, hence it was necessary to use the normal, lower concentration of phosphate. Equal amounts of inoculum were used and the cultures were prepared in duplicate. With the sugars, growth was measured nephelometrically with an Evelyn colorimeter, using the 520 filter, and with cellulose the percentage decomposition was measured. Results of the experiment are illustrated in Fig. 4.

VII. Inhibitors

Four compounds commonly used as inhibitors in the study of carbohydrate metabolism were tested for their effect on activity of the organisms, i.e., fluoride, iodoacetate, azide, and phlorhizin.

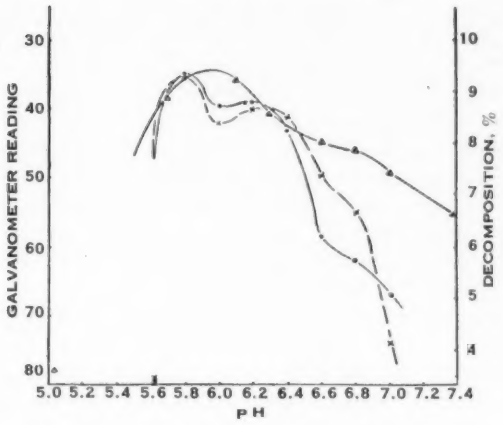

FIG. 4. *Optim..m hydrogen ion concentration for activity with cellulose, glucose, and cellobiose as substrates.*

△ – *Cellulose.*
× – *Cellobiose.*
○ – *Glucose.*

(*a*). *Effect on Respiration of Cellulose Cultures*

This was studied with the Warburg apparatus (direct method). No effect was apparent until the bacteria–cellulose suspensions had been incubated with the inhibitors for several hours. Results of an experiment in which oxygen uptake was measured after 12 hr. incubation are given in Table III. The concentration of inhibitor was 1% and the temperature was 27° C.

TABLE III

INHIBITION OF RESPIRATION OF A CELLULOSE CULTURE

(Oxygen uptake in cu. mm.; Temp. 27° C.)

Time, min.	Untreated	Fluoride	Iodoacetate	Azide	Phlorhizin
15	7.5	2.8	3.9	3.4	2.2
75	27.7	5.6	5.9	7.4	2.7
105	37.8	5.6	6.5	8.1	4.4
165	55.4	6.7	6.5	12.8	5.0

The respiration of the cellulose culture was inhibited in large measure in all cases.

(*b*). *Effect on Growth*

Cellulose, glucose, and cellobiose broths were used as the substrates. The concentration of the inhibitors was 0.5% for cellulose and 0.25% for the latter two. The temperature was 26.6° C.

In each case, with the exception of fluoride, complete inhibition resulted; in the presence of fluoride there was a slight growth in glucose and cellobiose broth. The untreated cultures all showed normal growth.

(*c*). *Effect on the Production of Glucose*

Azide and phlorhizin could not be used in these experiments because they interfere with the determination of sugars. The other two compounds were added in concentrations of 1% to aliquots of cultures treated with toluene in the usual manner, and reducing sugars determined periodically. Results are given in Table IV. Iodoacetate completely inhibited the formation of glucose, while fluoride was partially inhibitory.

TABLE IV

INHIBITION OF GLUCOSE FORMATION

(Mgm. glucose per 100 ml.)

Time, hr.	Untreated	Fluoride	Iodoacetate
13	12	Trace	0
19	25	12	0
38	43	18	0

Discussion

Aerobic, mesophilic bacteria have been characterized as yielding almost no products from cellulose other than carbon dioxide and synthesized cell materials (9). This also appears to be the case with the impure culture of *V. perimastix*. It has not been possible to determine the amount of carbon dioxide produced from cellulose by the bacteria, but it is obviously of a very high order since no other products appear in quantities large enough to explain the loss of cellulose.

The different rates of hydrolysis of the alcohol–acid precipitate and residual cellulose make it unlikely that the former is composed of short cellulose chains, but is possibly a synthesized bacterial polysaccharide. However, on hydrolysis the initial production of reducing substances from the precipitated material was rapid and then declined sharply, indicating that the material was not pure.

There is a striking similarity between the chemical and physical properties of the fluorescent pigment and riboflavin, so that the former is conceivably part of a flavin respiratory enzyme. If this is correct, the pigment would be expected to be present in large amounts in cultures showing rapid decomposition. The highest concentration of the pigment is obtained with alkali-treated cellulose, when the rate of decomposition is also most rapid.

Carbon dioxide has been found to be an essential growth factor for the bacteria and it is suggested that it plays some fundamental role in metabolic processes. Heterotrophic assimilation of carbon dioxide has been recognized

as an important process in the metabolism of several bacteria (15, pp. 135-182), and the list of organisms that require carbon dioxide continues to grow. This, however, appears to be the first instance to be reported of the essentiality of carbon dioxide in the case of aerobic cellulose-decomposing bacteria.

It is not known whether carbon dioxide is required for the breakdown of cellulose itself, or for the utilization of intermediate products. The carboxylation of pyruvic acid is the only known reaction that involves carbon dioxide assimilation (7), but this does not preclude other possibilities. Considering this reaction in the case of cellulose decomposition, the metabolic pathway of the polysaccharide would then, in part, coincide with that involved in the metabolism of many other carbohydrates.

The retarded growth observed in the presence of concentrations of carbon dioxide above that normally present in air may be due to the bacteria being adapted to lower carbon dioxide concentrations. In penicillin fermentations it has been found that varying the concentration of carbon dioxide affects the activity when the gas mixtures are bubbled through the cultures, but not when the cultures are merely shaken in an atmosphere containing the same gas mixtures (6). No explanation is given, but this observation indicates that the results may depend on the type of treatment.

There is a marked increase in the oxygen uptake with cellulose in the presence of carbon dioxide over that in its absence, which emphasizes the importance of carbon dioxide in the metabolism of the bacteria. The greater initial respiration rate in the absence of carbon dioxide is not explained.

The demonstration of the presence of appropriate dehydrogenases and the more rapid oxygen uptake with glucose would be in accord with the postulated role of glucose and cellobiose, respectively, as intermediates. Furthermore, the pH activity curves on glucose, cellobiose, and cellulose are somewhat similar, and glucose is also produced in fairly large amounts when the decomposition takes place in the presence of toluene. The increase in the respiration of an active cellulose-decomposing culture when cellobiose or glucose are added may also be significant. If the extra oxygen uptake is merely due to decomposition of the added glucose or cellobiose, then the respiration would be expected to follow a course similar to that with glucose or cellobiose alone. This is not the case, however, for the oxygen uptake in the absence of cellulose is greater with glucose than with cellobiose, and in the presence of cellulose the results are reversed.

These observations do not necessarily prove that glucose or cellobiose are normal intermediates in the decomposition of cellulose. Nevertheless, they strongly suggest a similarity in the metabolism of the bacteria on the three substrates. It is possible that phosphorylation mechanisms are involved, since phosphorylation inhibitors prevent growth on these carbohydrates, inhibit respiration of cellulose cultures, and retard the production of reducing sugars. In such a case, glucose, cellobiose, and cellulose could have common

pathways of dissimilation, and glucose and cellobiose could accumulate if the normal decomposition is disturbed, since they would be in equilibrium with other intermediates of cellulose decomposition. Stanier (13) has suggested that phosphorolytic decomposition is a possibility.

Glucose is produced in relatively large amounts when an active cellulose culture is treated with toluene. It is likely that a dialyzable factor, possibly a coenzyme produced by the bacteria, is involved in the production of glucose under these conditions. The formation of reducing sugars when toluene is present has been demonstrated with a number of other bacteria, but it has not previously been indicated that a dialyzable factor is involved. When the nature of this factor is known it may be possible to elucidate the mechanism of glucose formation, and possibly obtain a clearer insight into the nature of the enzymic processes involved in cellulose decomposition.

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A FORM OF *LEPTOSPHAERIA AVENARIA* ON WHEAT IN CANADA[1]

By T. Johnson[2]

Abstract

In the late summer of 1942, a *Septoria* sp. with spores longer than those of *Septoria nodorum* Berk., and conforming rather closely to those of *S. Avenae* Frank, was found commonly in the Prairie Provinces of Canada on leaves of wheat and, in one instance, on leaves of barley. It was found again on wheat and more rarely on barley in both Eastern and Western Canada each year from 1943 to 1946. Occasionally, perithecia, attributable to the genus *Leptosphaeria*, were found on leaves that previously had borne numerous pycnidia of this organism. That the perithecia represented the perfect stage of this *Septoria* sp. was shown by the development of similar perithecia in many cultures established from pycnidiospores, and by the formation of pycnidia in certain cultures grown from ascospores of perithecia collected on wheat. The perithecia, asci, and ascospores conform closely though not identically with the description of *Leptosphaeria avenaria* Weber.

The organism under discussion differs from *S. nodorum* not only in the size of its pycnidiospores but also in its inability to cause glume blotch, its longer incubation period, and lack of ability to attack the seedlings of certain wheat varieties susceptible to *S. nodorum*. It differs from *S. Avenae* in symptoms, host range, length of incubation period, and cultural characteristics. It differs from both these species by a lower parasitic vigour and a marked tendency to develop on fading leaves and sheaths. Owing to its morphological similarity to *L. avenaria* it is here described as a *forma specialis* of that species.

Introduction

During the late summer and fall of 1942, pycnidia of *Septoria* spp. were more than usually common on wheat leaves in the Prairie Provinces of Western Canada. In most of the samples examined by the writer, at least of those collected in Manitoba and Saskatchewan, the pycnidiospores did not conform to those of either *Septoria nodorum* Berk. or *S. Tritici* Desm. Pycnidia, though abundant on the leaves in many localities, occurred only rarely on the heads. The purplish-brown blotching of glumes and lemmas characteristic of wheat heads infected by *S. nodorum* was rarely seen although the extreme margins of the glumes were frequently brown and the terminal parts of glumes and lemmas often showed a faint, light brown discoloration. In some specimens of wheat examined, the pycnidiospores conformed closely to the description given for *S. nodorum* (1, 2, 5, 12) and were undoubtedly attributable to that species. In most specimens, however, the maximum length of the spores greatly exceeded the dimensions given for *S. nodorum*, the spores in many specimens reaching a maximum length of between 45 μ and 50 μ whereas the maximum length given for *S. nodorum* by any of the authors cited above is 36 μ. The spores were cylindrical, straight or slightly sinuate, rounded

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at the ends, three-septate or rarely four-septate, and in general, bore a resemblance in size and shape to the pycnidiospores of *Leptosphaeria avenaria* Weber (*Septoria Avenae* Frank) (see Figs. 2 and 3).

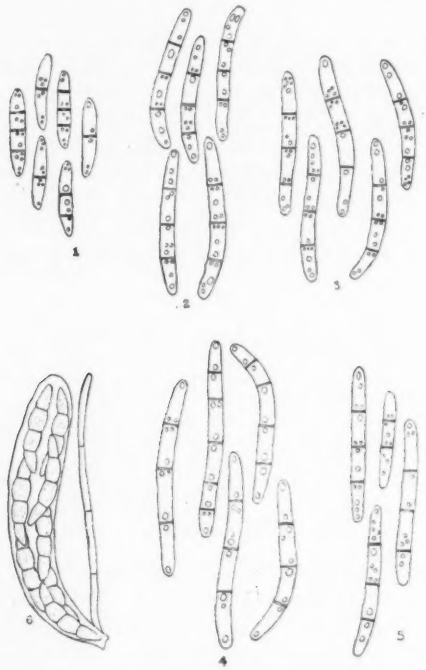

FIG. 1. *Septoria nodorum—pycnidiospores collected on wheat leaves at Kentville, N.S., 1946.*

FIG. 2. *Leptosphaeria avenaria f. sp. triticea—pycnidiospores collected on wheat leaves, Carlyle, Sask., 1946.*

FIG. 3. *Leptosphaeria avenaria f. sp. triticea—pycnidiospores collected on barley leaves, Morden, Man., 1945.*

FIG. 4. *Septoria Avenae—pycnidiospores collected on oats, Normandin, Que., 1946.*

FIG. 5. *Septoria Avenae—pycnidiospores collected on oats, Fort William, Ont., 1946.*

FIG. 6. *Leptosphaeria avenaria f. sp. triticea—ascus from a perithecium that developed in a culture originating from pycnidiospores that, in turn, developed in a culture grown from a perithecium collected on wheat at Wilcox, Sask., 1942.*

Magnification: Figs. 1 to 5, about 680 ×; Fig. 6, about 590 ×.

The same organism was again found, in 1943, on wheat specimens from both Western and Eastern Canada, but only in trace quantities. In 1944 it was widespread on wheat in Manitoba, but infection was mostly light though heavy infection was noted in several localities in the latter part of August. In that

year it was also found on wheat specimens in Ontario, Quebec, and the Maritime Provinces. In 1945 it caused widespread infection on wheat in Manitoba and the abundance of infection on fading leaves suggested that the death of the leaves was hastened by the infection. Slight to moderate infections were also noted in several localities in Eastern Canada. Infection of wheat in Manitoba was general but light in 1946. In that year, it occurred also at a number of places in the other two Prairie Provinces as well as in many localities in Eastern Canada.

Occurrence of the Perfect Stage

When pycnidia of this *Septoria* sp. were first observed in 1942, perithecia attributable to *Leptosphaeria* were occasionally found associated with them. This same association of pycnidia and perithecia was noted in several instances in 1943 and 1944. In the latter year, abundant perithecial development was observed at Winnipeg and at Gimli, Man. on wheat leaves bearing numerous pycnidia. At Winnipeg, perithecia were first seen on Aug. 25 on leaves of Gaza wheat. At that time they were still immature. On Sept. 7 the perithecia were very abundant and quite mature. Perithecia have also been found in 19 separate cultures derived from pycnidiospores, and pycnidia have been found in six different cultures derived from ascospores. The growth characteristics and pathogenicity of cultures originating from pycnidiospores and ascospores are so similar that it is clear beyond doubt that the perithecia represent the perfect stage of the *Septoria* sp.

Nomenclature

As information concerning this organism accumulated, the question of its nomenclature naturally arose. It was soon decided that it was readily distinguishable on the basis of the morphology of the pycnidiospores from *S. Tritici* and *S. Passerinii* Sacc. and from *S. graminum* either as originally described by Desmazieres* or as amended by Sprague (7); and is probably also distinguishable without difficulty from *S. Secalis* Prill. & Del., which, according to Weber (13), has somewhat narrower, curved pycnidiospores. It is separable from *S. nodorum* by the size and, to a certain extent, the shape of the pycnidiospores, and is also pathogenically different.

Of the wheat-inhabiting *Septoria* species the one that appears to resemble most closely the organism under discussion is *S. triticicola* Lobik, collected in the vicinity of Piatigorsk, U.S.S.R. (3). The symptoms and the dimensions of the pycnidia and spores agree approximately but the spores of the Russian species are described as three- to five-septate, slightly curved, and gradually tapering from base to tip, whereas the Canadian organism is generally three-septate with mostly straight spores that show little or no tapering. Furthermore, *S. triticicola* has no known perfect stage and so long as that is unknown it seems undesirable to identify the Canadian organism with it.

* *See Saccardo, P. A. Sylloge fungorum. III. Patavii. 1884.*

The organism here discussed is probably not morphologically distinguishable in either its perfect or imperfect stage from *Leptosphaeria avenaria* but is strikingly different pathogenically. The pycnidiospores appear also to be similar to those of *Stagonospora arenaria* Sacc., which according to Sprague (8), bear a close resemblance to those of *Septoria Avenae*. Although no cultures of *Stagonospora arenaria* were available for pathogenicity tests, that species is probably pathogenically distinct from the organism under discussion, which showed no ability, in green-house tests, to attack *Arrhenatherum elatius*, *Dactylis glomerata*, *Elymus canadensis*, or *Phalaris arundinacea*, which are reported to be hosts of *S. arenaria* (8).

The above considerations raise the question of where this *Septoria* sp. with its connected *Leptosphaeria* is to be placed taxonomically. There appear to be two alternatives. One is to describe it as a new species. Perhaps the chief objection to this course is that, if it were so described, it would be the only species of *Septoria* on cereals that could not be rather readily distinguished on a purely morphological basis. The other is to regard it as a *forma specialis* of the species it most resembles morphologically, namely, *Leptosphaeria avenaria*. As morphology rather than physiology is the basis of specific determination, the latter course seems the safest to follow. A somewhat similar course has been followed by Sprague who described (9) as *S. Tritici* Rob. f. *Avenae* (Desm.) Sprague, a narrow-spored type of *Septoria* on oats that he had originally (6) considered a physiologic form of *S. Tritici*. It is therefore suggested that the wheat-inhabiting *Septoria* here under discussion be regarded as a *forma specialis* of *Leptosphaeria avenaria*.

Description

Leptosphaeria avenaria WEBER, F. SP. *triticea* F. SP. NOV.

Maculis ovatis, saepe confluentibus, pallide luteolis, in media parte griseis. Peritheciis initio epidermide tectis dein erumpentibus, atris, globosis vel subglobosis, 100-220 μ dia., ostiolo circulari, 14-20 μ dia.; ascis clavatis, rectis vel paulum curvatis, hyalinis, 40-80 $\times$ 8-11 μ, octosporis, ascosporis biseriatis, fusoideis, rectis vel curvatis, 3-septatis, saepe ad septa constrictis, pallide luteolis (16) 19-25 (28) $\times$ 4-6 μ; paraphysibus filiformibus, septatis, paulo longioribus quam ascis.

FIG. 7. *Asci and spores of Leptosphaeria avenaria f. sp. triticea collected on Apex wheat leaves, Wilcox, Sask., 1942. About 495* $\times$.

FIG. 8. *Ascospores of L. avenaria f. sp. triticea formed on cornmeal agar. About 495* $\times$.

FIG. 9. *Young lesions of L. avenaria f. sp. triticea on Pelissier wheat, Winnipeg, Man., 1945.*

FIG. 10. *Cross section of pycnidium of L. avenaria f. sp. triticea collected on Gaza wheat, Aug. 18, 1942. About 295* $\times$.

FIG. 11. *Twelve-day old colonies of S. nodorum (left), L. avenaria f. sp. triticea (centre), and S. Avenae (right) on potato dextrose agar.*

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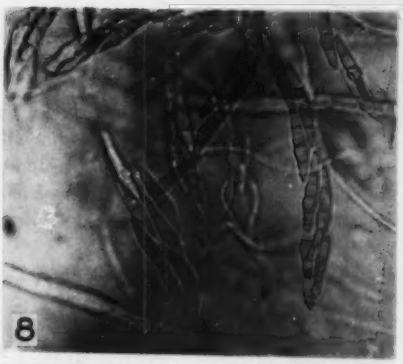
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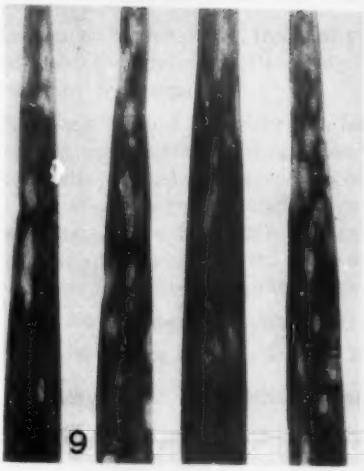
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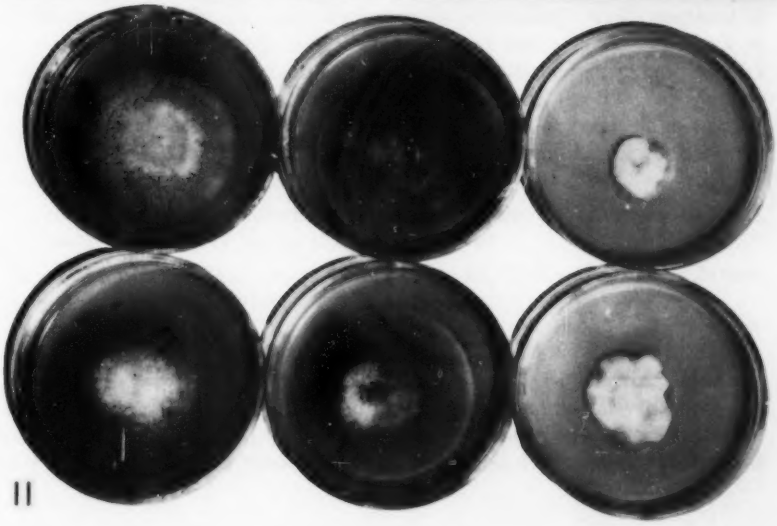
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STAT. PYCNID. *Septoria Avenae* FRANK F. SP. *triticea* F. SP. NOV.

Pycnidiis initio epidermide tectis dein erumpentibus, globosis vel subglobosis, atris (80) 90-140 (210) μ dia. cellis superficialibus polygoniis; ostiolo circulari vel ovato, 10-15 μ dia.; sporulis chlorino-hyalinis, cylindratis, rectis vel leniter sinuosis, utrinque obtusiusculis, 3-septatis vel raro 4-septatis (18) 26-42 (53) $\times$ (2.3) 2.8-3.5 (4.2) μ. Sporulis paulo brevioribus et angustioribus quam in *Septoria Avenae.*

Hab. in foliis vaginisque *Tritici vulgaris*, *Tritici duri* et rarius in foliis *Hordei vulgaris*, in Canada.

Leptosphaeria avenaria WEBER F. SP. *triticea* F. SP. NOV.

Lesions on leaves ovate, frequently coalescing, straw color or buff, central portion bearing pycnidia often gray. Pycnidia sometimes followed in late summer by perithecia.

Perithecia at first subepidermal, later erumpent, black by reflected light, brown by transmitted light, globose or subglobose, 100-220 μ in diameter, surface cells polyhedral, often pentagonal; ostiole circular 14-20 μ in diameter; asci clavate, straight or slightly curved, hyaline, 40-80 $\times$ 8-11 μ, eight-spored, ascospores biseriately arranged, fusoid, straight or curved, three-septate, frequently constricted at the septa, light yellow (16) 19-25 (28) $\times$ 4-6 μ; paraphyses filamentous, septate, slightly longer than the asci, hyaline.

On leaves and sheaths of wheat (*Triticum vulgare*, *Triticum durum*).

Type—Winnipeg, Man. Sept. 7, 1944. DAOM 19545.

PYCNIDIAL STAGE *Septoria Avenae* FRANK F. SP. *triticea* F. SP. NOV.

Pycnidia at first subepidermal, later erumpent, scattered, spherical or slightly elongate, black by reflected light, golden brown by transmitted light (80) 90-140 (210) μ in diameter, surface cells polyhedral; wall composed of three or four layers of brown cells, 7-11 μ thick at base and 11-14 μ thick at top of pycnidium; ostiole circular or oval, 10-15 μ in diameter; pycnidiospores hyaline to faintly chlorinous, thin-walled, cylindrical, straight or slightly sinuate, obtuse or rounded at the ends, three-septate or rarely four-septate, (18) 26-42 (53) $\times$ (2.3) 2.8-3.5 (4.2) μ. Spores generally somewhat shorter and more slender than in *Septoria Avenae.*

On leaves and sheaths of wheat (*Triticum vulgare*, *Triticum durum*) and, more rarely, on leaves of barley (*Hordeum vulgare*).

Type—Winnipeg, Man. Aug. 4, and Aug. 25, 1944 on *Triticum durum*, variety Gaza. DAOM 19546.

Host Range

Although *L. avenaria* f. sp. *triticea* is primarily a wheat-inhabiting organism, it is nevertheless found frequently on other cereals, particularly barley. Its presence was first noted on barley in 1942 when a bundle of heavily infected wheat from Indian Head, Sask. was found to contain two culms of barley, the

leaves of which bore numerous pycnidia. In 1943, pycnidia were found on barley leaves in 10 separate localities distributed in the provinces of Alberta, Saskatchewan, Manitoba, Ontario, Quebec, and Prince Edward Island. In no case, however, was the infection more than a mere trace. In 1944, the same organism was found on barley leaves at Winnipeg, Man., and in two localities in each of the provinces of Ontario, Quebec, and Nova Scotia. Infections were a mere trace except at Winnipeg where heavy pycnidial development occurred in August on the leaves of many barley varieties in the experimental plots. This infection, however, was somewhat lighter than in adjacent wheat plots. Barley was also found infected in 1945 and 1946 but never in more than trace amounts.

Less frequently than on barley, pycnidia of f. sp. *triticea* have been found on leaves and sheaths of oats, rye, and certain grasses, particularly species of *Elymus* and *Agropyron*, but as the pycnidia occurred chiefly on senescent plant parts it is doubtful whether these are congenial hosts. Examination and measurement of pycnidia and spores have shown the organism collected on these hosts to be morphologically the same as that collected on wheat and barley, and pathogenic tests have invariably shown that the cultures collected on oats and grasses as well as on barley attack wheat more readily than the host from which they were obtained. In fact, so rarely have infection tests with f. sp. *triticea* produced any symptoms whatsoever on barley, oats, or grasses that it seems probable that these hosts are highly resistant except when in a nearly mature or senescent condition.

Comparisons of *L. avenaria* f. sp. *triticea* with *Septoria nodorum* and *Septoria Avenae*

As soon as studies on *L. avenaria* f. sp. *triticea* were undertaken attempts were made to compare it morphologically and pathogenically with *Septoria nodorum* and *S. Avenae*. A few of many such tests are summarized in Table I.

Comparison of Morphological Characteristics

It was soon realized that f. sp. *triticea* and *S. nodorum* could not be distinguished by visual inspection of infected wheat leaves. Many specimens collected in Eastern Canada had both organisms present on the same leaves and mass scrapings from such leaves consequently contained spores of both. Scrapings from infected heads, however, almost invariably contained only the typical short-spored *S. nodorum* with pycnidia 80 to 140 μ in diameter and slightly curved, one- to three-septate, hyaline spores 15 to 25 $\times$ 2.5 to 3.5 μ. When pycnidia were detached singly from the leaves it was not possible to determine their specific identity until their spore contents were examined. It then became readily apparent that any given pycnidium contained either spores characteristic of *S. nodorum*, as described above, or spores of a somewhat different shape and considerably larger size. These spores were usually straight or slightly sinuous, cylindrical though often thickest at the base, three-septate, about 25-40 $\times$ 2.5-3.5 μ. Both as to dimensions and shape these spores were readily distinguishable from those of *S. nodorum*.

Evidence from comparisons of the pycnidial stage of *L. avenaria* f. sp. *triticea* with *S. Avenae* indicates that these two species cannot be distinguished readily on the basis of morphology. Over 200 measurements of pycnidia of f. sp. *triticea* collected on wheat and barley and about 60 measurements of pycnidia of pathogenically tested collections of *S. Avenae* gave the following dimensions (the extreme limits of size are shown in parentheses).

F. sp. *triticea* (80) 90-140 (210) μ. Ostioles mostly 10-15 μ.

S. Avenae (83) 110-150 (230) μ. Ostioles about 15 μ.

Pycnidia of both organisms were fuscous to black when seen with a lens but brownish yellow to light brown when viewed with a microscope.

Measurements of spores from 47 samples of f. sp. *triticea* collected on wheat, and 24 samples of *S. Avenae* gave the dimensions below:

f. sp. *triticea* (18) 26-42 (53) $\times$ (2.3) 2.8-3.5 (4.2) μ.

S. Avenae (21) 29-45 (61) $\times$ (2.5) 2.9-3.9 (4.5) μ.

These data indicate that both pycnidia and spores of *S. Avenae* are slightly larger than those of f. sp. *triticea* but in view of the similarity in appearance of the pycnidia and of the spores of the two organisms, the size differences are insufficient to permit of ready distinction between them.

Comparison of the perfect stage of f. sp. *triticea* with that of *S. Avenae* is rendered difficult by the fact that the perithecia of *S. Avenae* (*Leptosphaeria avenaria*) have not been found on field material and are known only from Weber's description (11) of perithecia and ascospores formed in cultures growing on agar. The following is a comparison of the dimensions of perithecia, asci, and ascospores of *L. avenaria* f. sp. *triticea* with corresponding dimensions of *L. avenaria* given by Weber.

L. avenaria f. sp. *triticea*

Perithecia (in nature) 110-150 (220) μ. Ostiole 14-20 μ.

Perithecia (in culture) about 140 (220) μ.

Asci (in nature) (42) 50-70 (78) $\times$ (7) 8-10 (11) μ.

Asci (in culture) (36) 50-70 (85) $\times$ (6) 8-11 μ.

Ascospores (in nature) (16) 19-25 (27) $\times$ (3.9) 4.5-5.5 (6.0) μ.

Ascospores (in culture) (16) 19-25 (28) $\times$ (3.0) 4.0-5.0 (5.3) μ.

L. avenaria (according to Weber)

Perithecia (in culture) 60-130 μ. Ostiole 12-20 μ.

Asci (in culture) 30-100 $\times$ 10-18 μ.

Ascospores (in culture) 23-28 $\times$ 4.5-6.0 μ.

Weber's data indicate for *L. avenaria* somewhat smaller perithecia and wider asci and ascospores than in *L. avenaria* f. sp. *triticea* but it is doubtful whether much significance should be attached to these differences.

Comparison of Pathogenic Characteristics

In host range and symptoms f. sp. *triticea* and *S. Avenae* are quite distinct. The latter, which is actively parasitic on oats, produces rather large chocolate-brown or purplish-brown, oval lesions in which pycnidia are present though usually not readily visible against the pigmented lesion.

F. sp. *triticea* has been found to occur on oats but in all the known instances it was present on plant parts (leaves or upper parts of sheaths) that had reached maturity and was not accompanied by the characteristic brown lesions of *S. Avenae.* It is evidently not actively parasitic on oats although it is possible that it can become established on fading plant parts.

Comparative tests were made of the pathogenicity of f. sp. *triticea*, *S. nodorum*, and *S. Avenae* by infecting seedling plants of wheat, oats, barley, and rye. In these tests two different methods were used: (1) Infection with spore suspensions obtained directly from infected plants gathered in the field. (2) Infection with pure cultures of the organisms, each culture commonly established from the spores of a single pycnidium.

The two methods produced similar results but the first (the use of spores gathered directly from field material) had the disadvantage that other parasitic organisms were sometimes present on the field material from which the spore suspensions were secured. The majority of the infection tests were therefore carried out with pure cultures of monopycnidial origin, the inoculum being produced on finely ground oat hulls. The ground oat hulls were placed in test tubes, moistened with water, autoclaved, and inoculated with spores or mycelium. Later, when the mycelial growth had penetrated to some depth in the tubes, part of the contents was removed, macerated in water, and applied to the leaves with the fingers. Following inoculation, the seedling plants were kept in moist chambers for a period of two or three days. By this method it was found that the pathogenicity of even non-sporulating cultures could be tested readily.

F. sp. *triticea* proved pathogenically much weaker on wheat than did *S. nodorum*, which normally destroyed inoculated leaves in six or seven days. In that time, f. sp. *triticea* produced scarcely any visible symptoms. Four or five days later, however, numerous necrotic areas usually developed. These generally took the form of small oval lesions of buff color, sometimes with a darker centre. A large number of infection experiments has indicated that the incubation period of f. sp. *triticea* is about twice as long as that of *S. nodorum*, or approximately 10 days while that of *S. nodorum* is five days.

F. sp. *triticea* differs furthermore from *S. nodorum* by its inability to attack, in the seedling stage, certain wheats highly susceptible to the latter. The virtual immunity of seedling leaves of the varieties McMurachy and Regent from attack by f. sp. *triticea* proved a useful means of distinguishing that species from *S. nodorum*, which attacks these varieties vigorously.

Neither f. sp. *triticea* nor *S. nodorum* was able to attack seedlings of barley, rye, or oats. The inability to attack oats distinguished these species sharply from *S. Avenae*, which was extremely pathogenic to oat seedlings on which it produced typical, oval, brown lesions in four or five days after inoculations (Table I).

TABLE I

PYCNIDIOSPORE DIMENSIONS AND PATHOGENICITY ON CEREAL SEEDLINGS OF *Leptosphaeria avenaria* F. SP. *triticea*, *L. avenaria*, AND *Septoria nodorum*

Culture No.	Source		Spore size, μ		Pathogenicity (% of terminal three inches of seedling leaf killed)					
			Range	Mean length	Wheat			Oats	Barley	Rye
					Gaza	Marquis	Regent			
	L. avenaria f. sp. *triticea*—from pycnidia on wheat									
2	Winnipeg, Man.	1942	28-42 × 3.0-3.6	34	8	6	0	0	0	0
103*	" "	1943	28-49 × 2.6-3.3	37	7	4	3	0	0	0
142	" "	1944	27-42 × 2.8-3.6	34	19	5	0	0	0	0
143*	Morden, Man.	1944	29-43 × 3.1-3.6	36	6	5	1	0	0	0
150	Guelph, Ont.	1944	22-44 × 2.8-3.6	30	33	14	6	0	0	0
233	Indian Head, Sask.	1945	22-41 × 2.8-3.6	34	2	1	0	0	0	0
225	Kemptville, Ont.	1945	20-37 × 3.1-3.3	30	5	6	0	0	0	0
385	Kapuskasing, Ont.	1945	39-50 × 2.8-3.3	44	12	5	0	0	0	0
	L. avenaria f. sp. *triticea*—from perithecia on wheat									
33*	Wilcox, Sask.	1942			2	1	0	0	0	—
46*	" "	1942			5	8	0	0	0	—
157	Winnipeg, Man.	1944	Ascospores 20-25 × 4.4-5.6		54	17	6	0	0	0
158*	" "	1944			8	5	1	0	1	0
161	" "	1944			16	10	0	0	0	0
173	Gimli, Man.	1944	Ascospores 22-25 × 5.6		16	3	0	0	0	0
	L. avenaria f. sp. *triticea*—from pycnidia on barley									
12	Indian Head, Sask.	1942	20-38 × 2.6-3.3	26	7	5	2	0	0	0
100	Winnipeg, Man.	1943	24-35 × 2.4-3.0	29	14	0	0	0	0	0
141*	" "	1944	28-40 × 2.8-3.3	34	6	4	1	0	0	0
228	" "	1945	30-42 × 2.8-3.9	37	3	1	0	0	0	0
320	St. Norbert, Man.	1946	28-40 × 2.8-3.3	35	tr.	0	0	0	0	
	S. nodorum									
77	Wilcox, Sask.	1942	17-24 × 3.0	21	34	55	60	0	0	0
113	Ottawa, Ont.	1943	17-27 × 2.4-3.7	23	46	41	36	0	1	0
151	Guelph, Ont.	1944	17-28 × 2.8-4.2	20	54	49	52	0	0	0
153	Lennoxville, Que.	1944	17-28 × 2.8-3.3	20	70	76	83	0	0	0
204	Winnipeg, Man.	1945	20-32 × 2.8-3.3	27	46	42	55	0	tr.	0
360	Fredericton, N.B.	1946	18-22 × 2.8-3.3	20	40	35	54	0	0	0
	L. avenaria									
176	Fort William, Ont.	1944	24-40 × 3.1-4.5	34	0	0	0	45	0	0
238	Kapuskasing, Ont.	1945	28-39 × 2.8-3.6	33	0	0	0	18	0	0
213	St. Catharines, Ont.	1945	34-48 × 3.1-3.6	41	0	0	0	52	0	0
232	Guelph, Ont.	1945	27-35 × 2.8-4.2	30	0	0	0	6	0	0
362	Kentville, N.S.	1946	25-43 × 2.8-3.3	36	0	0	0	8	0	0
375	Normandin, Que.	1946	31-46 × 2.8-3.9	40	0	0	0	6	0	0
382	Kapuskasing, Ont.	1946	36-61 × 2.5-3.9	49	0	0	0	6	0	0

* *Perithecia formed in culture.*

Comparison of Cultural Characteristics

Tests on potato dextrose, cornmeal, and malt agar have shown that f. sp. *triticea* differs but little from *S. nodorum* in growth characteristics but may be distinguished from *S. Avenae* rather readily.

Both f. sp. *triticea* and *S. nodorum* produce at first a fine-textured, creamy-white, cottony mycelium that later assumes a buff color. The aerial mycelium of f. sp. *triticea* is usually rather coarser in texture, more tufted, and the substratum shows a more marked tendency to develop a dark-green or bluish-green pigment. Despite these apparent differences, cultures of the two organisms are not always readily distinguishable owing, largely, to the considerable variation between different cultures of the same species. Both organisms normally produce round colonies with uniformly smooth edges.

Colonies of *S. Avenae* are definitely slower in growth, and generally produce a cushiony aerial mycelium of a pure white color with little or no pigmentation in the substratum. The margins of the colonies are commonly ragged or irregular in appearance (Fig. 11).

Discussion

There is no reason to suppose that the organism here described as *Leptosphaeria avenaria* f. sp. *triticea* has made its appearance only in the last few years. Examination of herbarium specimens has proved the presence on wheat in past years of the pycnidial stage of this species. One such specimen collected on wheat at Winnipeg in 1927 was provisionally identified as *Septoria Tritici*, presumably because the pycnidiospores were too long to fit the description of *S. nodorum*. Another similar specimen was collected on wheat at Rosthern, Sask., in 1928 by Dr. R. C. Russell and submitted to Dr. G. R. Bisby for identification. The latter labelled the specimen *S. nodorum* but noted that the dimensions of the spores were 25.2-43.2 × 3-4 μ. Dr. R. Sprague also assigned to *S. nodorum* a large-spored type of *Septoria* probably similar to the one under discussion (10). The pycnidial stage of *L. avenaria* f. sp. *triticea* may also have been attributed by some collectors to *Stagonospora arenaria* Sacc. the pycnidiospores of which, according to Sprague (8), bear a close resemblance to those of *Septoria Avenae*, which in turn resemble those of f. sp. *triticea*.

The reason why f. sp. *triticea* has not been previously recorded as a separate entity lies perhaps in the inconspicuous nature of the symptoms, a certain resemblance of the pycnidia and spores to those of *S. nodorum*, and the fact that the two organisms sometimes occur together on the same plant.

The disease caused by f. sp. *triticea* is probably of minor economic importance. In 1942, 1944, and 1945 it caused considerable leaf necrosis on wheat in Manitoba but as this occurred rather late each season it is doubtful whether it led to much reduction in yield. The organism appears to develop best on leaves approaching the senescent stage, a fact that, taken in conjunction with the slow and, in comparison with *S. nodorum*, limited development of necrotic

areas in greenhouse tests, would indicate a relatively weak parasitism. Its long incubation period no doubt slows up its development in early and mid summer and probably prevents the appearance of any sudden epiphytotics.

That pathogenicity on barley is of a still lower order is shown by the rare development of symptoms on barley seedlings in greenhouse tests and by the absence in the field of heavy or even moderate attacks except in late summer on leaves already senescent.

L. avenaria f. sp. *triticea* does not ordinarily produce visible symptoms on the heads of either wheat or barley. There is, nevertheless, evidence that infection of the heads of both cereals does take place. During the course of the work here reported Dr. J. E. Machacek turned over to the writer many cultures of undetermined *Septoria* species isolated from seed of wheat, barley, and oats grown in various parts of Canada (4). These were found to fall into two classes (i) typical *S. nodorum*, collected most frequently in Eastern Canada, and (ii) f. sp. *triticea*, most frequently collected in Western Canada.

From Dr. Machacek's extensive isolation of *Septoria* cultures from wheat and barley seed collected in all the provinces of Canada over the period 1939-1943 it is evident that *S. nodorum* shows a strong tendency to infect wheat seed in preference to barley seed. In the Maritime Provinces, where that species is common, *Septoria* cultures (species not always determined) were isolated 26 times more frequently from wheat seed than from barley seed. In the Prairie Provinces where f. sp. *triticea* is common, *Septoria* cultures were isolated twice as often from barley seed as from wheat seed, which indicates that the hull of the barley seed is a favorable medium for the establishment of the fungus, perhaps saprophytically. The occurrence of f. sp. *triticea* on seed is probably a reflection of its tendency to invade mature tissues. Whether such invasion constitutes a means of overwintering for the organism is not certain. Greenhouse studies have shown that coleoptile lesions will occur on plants growing from infected seed but there is little or no proof of further spread to the growing plant.

Acknowledgments

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Phosphorus Deficiency in Relation to the Nitrate Reduction Test

In classifying bacteria, the ability to reduce nitrates to nitrites is an important characteristic. The *Manual of Methods for the Pure Culture Study of Bacteria* (1) recommends two media, one for organisms requiring organic nitrogen, the other for soil bacteria and related organisms not needing organic nitrogen.

Both media have been used in this laboratory in testing certain soil bacteria, long suspected of being capable of nitrate reduction, although they often show negative results. In addition to these organisms, a group of closely related cultures received from H. L. Jensen of New South Wales, including some so-called 'Corynebacteria' from the soil, were tested using the medium recommended by Jensen (2) (see Table I). A much higher percentage of positive tests was found with this medium on all cultures than with the synthetic 'Manual' medium.

TABLE I

SYNTHETIC NITRATE MEDIA

	Manual of methods	Jensen's medium	Proposed medium
K_2HPO_4	0.5 gm.	1.0 gm.	0.5 gm.
$CaCl_2$ (anhyd.)	0.5 gm.	—	—*
NaCl	—	0.5 gm.	0.5 gm.
$MgSO_4 + 7\ H_2O$	0.2 gm.	0.5 gm.	0.2 gm.
$NaNO_3$	—	2.0 gm.	2.0 gm.
KNO_3	1.0 gm.	—	—
Glucose	10.0 gm.	10.0 gm.	10.0 gm.
Agar	15.0 gm.	20.0 gm.	15.0 gm.
Distilled water	1000.0 cc.	1000.0 cc.	1000.0 cc.

* *If it is suspected that the organisms being studied require more calcium than is furnished by impurities present in the other ingredients, a small amount (not over 0.05 gm.) calcium chloride may be added.*

With this in mind, a series of media were made up in order to determine, if possible, why Jensen's medium was superior. Using the 'Manual of Methods' medium as a basis, successive changes of one ingredient at a time were effected to make it, step by step, more like Jensen's medium. A change from potassium to sodium nitrate or from calcium to sodium chloride showed the greatest increase in the number of positive results. A new medium (see Table I, last column) was then tested with the media recommended in the Manual, using 65 organisms that included four spore-formers, three *Actinomyces* strains, 30 cultures showing the morphology typical of *Bacterium globiforme*, 23 other soil forms, three '*Corynebacterium*' cultures obtained from Jensen, and four

miscellaneous types. The new medium gave far superior results, that is, there were a greater number of nitrate positive organisms and a much smaller number of nitrate negative ones.

In order to determine why the new medium was better than that of the Manual of Methods, further tests were carried out. Because the greatest difference between the two media lay in the cations of the nitrate and the chloride, it appeared that the presence of sodium enhanced the production of nitrite in some way. Results, obtained when the amount of sodium or potassium was varied or when calcium was replaced by either one, indicated that the presence of both cations enhanced nitrate reduction even when calcium was used; but the best results were secured when sodium or potassium was substituted for calcium. It was noted that some precipitation of phosphate always occurred in the medium containing calcium, even when precautions were taken (as directed in the Manual) to dissolve the calcium salt and the phosphate separately; it was suspected, therefore, that phosphorus deficiency might be the cause of poor results obtained with it. To investigate this point, the same medium was made up without any phosphate, but with all other ions present; as was expected, growth was scanty and the percentage of positive reactions was very low.

It is apparent then that two factors are involved in the superiority of the proposed medium: first, both sodium and potassium ions are essential; second, too high a concentration of calcium precipitates the phosphates in the medium (as is visible in the preparation of the medium), thereby *interfering with nitrate reduction, even though enough phosphate remains available to permit what seems to the eye to be normal growth.* Complete elimination of the calcium ion is unnecessary as 0.05 gm. of calcium chloride per litre provides calcium but does not interfere with nitrate reduction. Inasmuch, however, as experiments show that the organisms included in this work do not need more calcium than they may be given in the form of impurities of the distilled water or 'chemically pure' salts, there seems to be no need for including it in the formula.

Accordingly, it is recommended that the synthetic nitrate medium of the Manual of Methods be replaced with the formula given in the last column of Table I, or at least with some similar formula calling for both sodium and potassium salts, but no calcium in any form. It must, of course, be recognized that this recommended medium, like the synthetic formula in the Manual of Methods is applicable only to those organisms that do not require organic nitrogen.

A search through the literature shows that many other media have been recommended for one purpose or another that calls for some phosphate together with comparatively large amounts of calcium. It is quite possible that an investigation of these media would show that bacteria growing in

them, also, suffer from phosphate deficiency sufficiently to interfere with their physiology, even though (like the organisms here investigated on this nitrate medium) their growth appears normal to the eye.

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